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BULLETIN OF THE TORREY BOTANICAL CLUB
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Fundamental developments in the field of plant growth regulators

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Since the discovery of the compound indoleacetic acid about 20 years ago, we now have more than 1,000 organic chemicals that have plant growth-regulating properties. We have studied these regulators, which are considered a class of biologically effective compounds, by first observing the many ways that plants respond to them. We then have attempted to explain on a chemical or biochemical basis the mechanism of action that accounts for each response. Many discoveries of practical importance have resulted mainly from the study of plant responses to regulators (VanOverbeek 1952, Wain and Wightman 1956).

The objective in this report is to review some of the major developments in this response type of research, to consider current developments in this field and to point out some agricultural problems that lend themselves to research with these chemicals[1].

CELL ENLARGEMENT AND MULTIPLICATION. Chemically-induced cell enlargement represents the first major step toward understanding how growth and behavior of plants can be controlled with regulating chemicals. It is believed that this cell reaction involves changes in the wall that allow the cell to absorb water and increase in size. Various investigators have suggested that loosening of the wall material, the deposition of new wall substances or non-osmotic water intake may occur in a cell under influence of the regulator. Any of these wall responses might increase water movement into the cell and partly account for cell enlargement (Bonner, Ordin and Cleland 1956, Brauner and Hasman 1952, Burström 1953, Cleland and Bonner 1956, Hackett 1952, Kobayashi, Hatakeyama and Ashida 1956, Levitt 1948, Ordin, Applewite and Bonner 1956, Thimann 1951 and Went and Thimann 1937). Indoleacetic, 4-chlorophenoxyacetic, naphthaleneacetic and 2,4-dichlorophenoxyacetic acids are among the regulators that induce cell enlargement associated with water intake. An increase in the water-retaining capacity of cells that responded experimentally to these chemicals was observed when plants were sprayed with 4-chloro-

[1] Since this review covers developments that stimulated the publication of thousands of technical articles in the field of regulating chemicals, only a few references pertaining to the early research connected with each development can be cited.

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phenoxyacetic acid (Mitchell and Marth 1950). The enlarged cells of bean pods, for example, retained moisture more readily than did untreated ones under experimental conditions that favor relatively rapid evaporation (fig. 1).

Fig. 1. Moisture retention—turgid bean pods from plant sprayed 4 days before harvest with 4-chlorophenoxyacetic acid (right), compared with pods from unsprayed plant. All pods were removed from plants at the same time then allowed to dry for 48 hours.

Our knowledge of responses was advanced when it was learned that certain cells in the stems of plants could be stimulated to divide by use of relatively large amounts of regulators such as indoleacetic, indolebutyric and naphthaleneacetic acids. Cell multiplication induced by these compounds in stems was followed by maturation of new cells to form tissues and finally their organization into root primordia (Hamner and Kraus 1937; Hitchcock 1935; Zimmerman, Hitchcock and Crocker 1931; and Zimmerman and Wilcoxon 1935).

Cells stimulated in this manner are able to mobilize, from other areas of the plant, endogenous and, incidentally, some translocatable exogenous substances that might be present (Mitchell and Linder 1957). Both organic and inorganic constituents of the plant are involved in this migration of substances toward the chemically-induced center of metabolic activity. Included is the mobilization of minerals such as potassium, magnesium, boron, copper, phosphorus, iron and aluminum (Brunstetter, Myers, Mitchell, Stewart and Kaufman 1948). Various nitrogenous compounds

and carbohydrates are also strongly attracted to the site of induced metabolic activity (Stuart 1938).

These primary responses represent early steps in the development of our general understanding of how plant growth and behavior can be controlled with regulating chemicals. Chemically-induced mobilization of the kind described is thus far of no practical use. Chemical induction of root primordia, on the other hand, led directly to use of these compounds in the vegetative propagation of many plants.

Recent developments concerning methods of vegetative propagation include the use of a very active regulator, 2,4,5-trichlorophenoxyacetic acid, as a stimulant for hard-to-root cuttings such as some species of rhododendron (Wells and Marth 1954). Even with the root-inducing chemicals available, some important economic plants still cannot be readily propagated vegetatively.

ABSCISSION. Indoleacetic acid placed on debladed *Coleus* petioles retarded abscission so that the petioles remained attached to the stems longer than did untreated ones (LaRue 1936). This observation represented an important fundamental advance in our knowledge of growth-regulator response. The effect was later obtained with holly berries (Gardner and Marth 1937) and apples (Gardner, Marth and Batjer 1939), and the response is now used to improve the quality of apples and pears in the United States. Abscission of relatively mature fruits involves dissolution of some wall constituents (MacDaniels 1936; McCown 1943; and Sampson 1918), a process that can be directly or indirectly retarded with regulators (Skoog 1951). According to Bonner (1936), abscission of this type involves breakdown of the middle lamella. An entirely different process, one that involves rapid stem growth under conditions of limited carbohydrate supply, may account for the drop of apples that commonly occurs during an earlier stage in their development (VanOverbeek 1952). A problem not yet solved in this field is the need for a regulator that retards abscission of early varieties of fruit without acceleration of ripening.

In contrast to retarded abscission, the opposite effect can be obtained. Some leaves and fruits can be made to fall earlier by use of a chemical. Shull (1930) demonstrated about 30 years ago that leaves of rose plants can be made to fall off with ethylene. This was at a time when growth regulators in a solid state were still unknown.

Regulators such as naphthaleneacetic acid that now afford a means of thinning fruit are thought by some to deprive cells at the base of the very young fruit stem of carbohydrates needed to build cellulose walls with sufficient strength to support the weight of the rapidly developing fruit (VanOverbeek 1952). Seed abortion may also play a role in the thinning action of this regulator (Luckwell 1953). Batjer and Westwood (1960) recently found that 1-naphthyl N-methylcarbamate (an insecticide) en-

hanced the drop of immature apples of some varieties. Use of this insecticide represents a new method of chemical thinning that is of interest in some areas where apples are grown. Seed abortion occurred when some varieties of apples were sprayed with this chemical.

FRUIT GROWTH. An important addition to our knowledge of responses to regulators was made by Gustafson (1936), who demonstrated that some regulating chemicals induce growth of fruits when applied to the unpollinated flowers. The first effect of the chemical is to prevent abscission of well-developed flowers, and the second is to stimulate growth of fruits. Ordinarily these are functions of hormones produced by the seeds following pollination and fertilization (Nitsch 1950 and Luckwell 1957). Most recent research in this field involves parthenocarpic development of almonds, apricots and peaches (Crane, Primer and Campbell 1960).

Hormones produced by seeds as they develop may sometimes limit fruit growth. This concept is based on the fact that under some conditions the growth of fruits such as tomato, orange and others can be accelerated with synthetically-produced regulators (Erickson and Brannaman 1950 and Stewart, Klotz and Hield 1951).

Regulating substances also influence physiological processes involved in maturation of fruits in addition to those concerned with the early development of fruits. For example, ripening of detached bananas and other fruits can be hastened with ethylene and 2,4-D (Mitchell and Marth 1944). Ripening of figs, apples and peaches still attached to the tree can be accelerated; but no practical use of this response has yet been made (Blondeau and Crane 1948; Harley, Marth and Moon 1950; and Marth, Havis and Prince 1950).

REGULATORS AS HERBICIDES. In 1942 it was discovered that some chlorinated phenoxyacetic acids possessed growth-regulating properties (Zimmerman and Hitchcock 1942). Later, it was realized that even relatively small amounts of these compounds induced systemic physiological responses that resulted in death of plants (fig. 2) (Kraus and Mitchell 1947 and Slade, Templeman and Sexton 1945).

The primary reaction resulting from the use of lethal amounts of growth-regulating compounds of the phenoxy type is not understood. Results of use of indoleacetic acid indicate that energy transfer involving phosphorus metabolism may be concerned, and that changes in organic acid metabolism may be a primary response (Skoog 1951 and VanOverbeek 1952). Many responses to 2,4-dichlorophenoxyacetic acid, apparently of a secondary nature, have been noted indicating that numerous enzymatic and possibly some nonenzymatic processes are altered. Some readily detectable secondary effects include an increase in the moisture content of tissues affected, hydrolysis of reserve carbohydrates, depletion of sugars and proliferation of responsive parenchymatous cells (Hamner and Kraus

1937; Mitchell and Brown 1945; and Smith, Hamner and Carlson 1947). It has been suggested that the accumulation of unusually large amounts of toxic metabolites may also be involved (VanOverbeek, Blondeau and Horne 1951). Hilton and his coworkers (1959) showed that some chloro-substituted aliphatic acids, including the herbicide Dalapon, compete with the natural substrate involved in the enzymatic synthesis of pantothenate. They believe that this interference with enzyme activity may account for lethal effects of these acids.

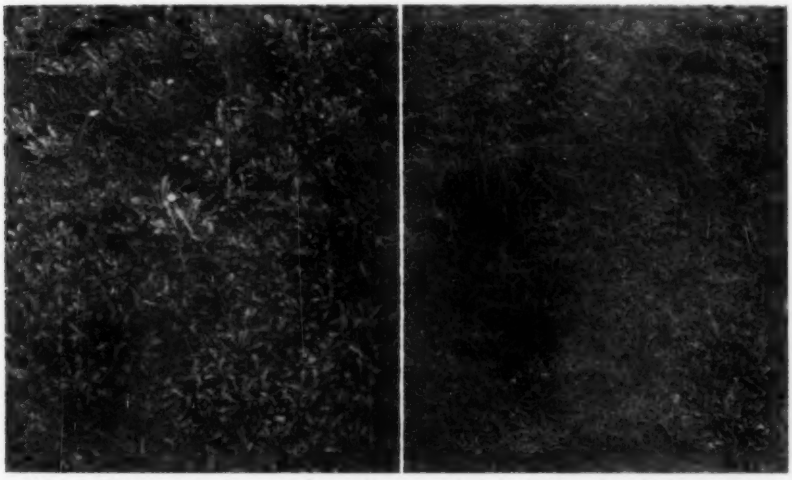

Fig. 2. Photograph of one of the earliest experiments (1944) that demonstrated the possibility of using the growth regulator 2,4-dichlorophenoxyacetic acid for selective control of dandelions in turf. Sprayed plot on right.

Intensive basic research (Danielson, Gentner and Jansen 1960; Gentner and Hilton 1960; Hilton, Ard, Jansen and Gentner 1959; Jansen, Gentner and Shaw 1960; and Moreland, Gentner, Hilton and Hill 1959) in the field of herbicides is in progress, including studies on the role of carriers, absorption and translocation, the influence of structure, and mechanism of action. This line of research parallels similar research in the field of regulating chemicals. Together, these efforts should soon lead to a much more complete understanding of how compounds regulate the growth of plants and how they kill them.

RESPONSES TO GIBBERELLINS AND GIBBERELLIN-LIKE HORMONES. Only recently has it been possible to accelerate with growth regulators the rate at which some plants gain weight. A number of investigators have obtained this response by applying gibberellic acid to various kinds of plants (Brian, Elson, Hemming and Radley 1954; Ergle 1958; Howell et al. 1960; Humphries and French 1960; Marth, Audia and

Mitchell 1956; Stuart and Cathey 1958; and Wittwer and Bukovac 1958). Overall growth stimulation with gibberellic acid apparently involves, for one thing, an accelerated rate of carbon assimilation which is thought to result from increased leaf area rather than from increased photosynthetic efficiency per unit of leaf area (Haber and Tolbert 1957 and Stowe and Yamaki 1957).

Elongation is the initial growth response to this regulator (Stowe and Yamaki 1957). Increased cambial activity has been observed as a secondary effect, however, with the result that thickening of stems finally occurred when perennial plants, such as long leaf pine and walnut, were treated with the acid and observed under greenhouse conditions (Agricultural Research Service 1958). Gibberellic acid experimentally reduced the rest period required by the vegetative buds and seeds of some plants (Stowe and Yamaki 1957). Increased production of amylase by barley seeds subjected to gibberellic acid is now of practical importance in malt production (Bawden, Dahlstrom and Sfat 1959; Munekata and Kato 1957; and Sandegren and Beling 1959).

It was demonstrated repeatedly during the last 10 years that there are naturally occurring compounds in higher plants that can induce responses similar to those induced by gibberellic acid, such as stem elongation and leaf growth. Readily detectable amounts of these substances are present in various kinds of pollen, seeds and plant parts (Mitchell, Skaggs and Anderson 1951, Radley 1956 and West and Phinney 1956). The function of these extractable hormones is not known. One of these, obtained from the endosperm of bean seeds of the Black Valentine variety, was applied to immature pods on plants of the same variety. The pods did not respond measurably, perhaps because they already contained a sufficient amount of hormone or they were insensitive to it. The same hormone, however, accelerated growth when applied to the stems and leaves of bean plants and it no doubt plays a role in the growth and development of bean plants.

GROWTH RETARDATION. Another advance in regulator research came in 1949 (Mitchell, Wirwille and Weil) when it was learned that some chemicals can be used to retard overall growth without apparent injury to the plant (fig. 3). Several compounds are now known to induce this response (Marth and Mitchell 1960). There is evidence that the retardant Amo-1618 inhibits cell division in the subapical region of stems in some caulescent plants and in this way retards growth (Sachs 1959). Growth retardation with maleic hydrazide may also depend largely on interference with cell division (Haber and White 1960). Although about 10 years has elapsed since the discovery of growth retardants, we have relatively little knowledge regarding the histological, cytological and biochemical processes involved when the overall growth of plants is controlled in this way.

There is a different type of retardation, however, one that involves complete inhibition of vegetative bud growth rather than partial retarda-

Fig. 3. Retardation of growth of raspberry plant sprayed with (4-hydroxy-5-isopropyl-2-methylphenyl) trimethyl ammonium chloride, 1-piperidinecarboxylate (right) compared with the growth of an untreated raspberry plant of the same kind (left.).

tion of the entire plant. This type of bud inhibition was first observed by Guthrie (1939) who noted that vapors from the methyl ester of naphthaleneacetic acid prevented growth of buds on potato tubers. More recently maleic hydrazide and 3-chloro-isopropyl-N-phenyl carbamate have been used for this purpose (Marshall and Smith 1951 and Marth and Schultz 1952). Templeman and Sexton (1945) observed that isopropyl phenyl carbamate modified the ability of cells to divide in a normal pattern, and this may account for its growth-retarding properties. Chemical suppression of vegetative bud growth has, of course, proved of practical value, especially in the potato industry (Heinze, Marth and Craft 1955).

DEVELOPMENTS CONCERNING ABSORPTION AND TRANSLOCATION OF REGULATORS. To be effective, regulating chemicals must, of course, be absorbed by the plant and then translocated to the site of action. Considerable research has been directed toward gaining an understanding of these processes. (Mitchell, Smale and Metcalf 1960).

It was discovered almost 15 years ago that movement of regulators from leaves to other parts of plants depends on movement of photosynthate in the same direction (Hay 1955, Mitchell and Brown 1946, Rice 1948 and Weaver and DeRose 1946). Although this early development represents a step toward understanding translocation of regulators, the role of photosynthate in the process is still not understood.

Hamner, Lucas and Sell (1947) learned that acid solutions of some regulators were more effective than the same ones made alkaline. An important development was the use of adjuvants to increase the effectiveness of regulators (Zimmerman and Hitchcock 1942). Effects of adjuvants are most marked when minute amounts of regulating compounds are used.

Currently, the relationship between molecular configuration and the ability of plants to absorb and translocate regulators is being studied. The marked influence of a methoxy group or a methyl group when associated with the alpha carbon in the carboxylic-acid side chain of some regulators has been demonstrated. Substitution of a methyl group for a hydrogen atom of an alpha carbon may influence metabolism of the regulator. Naphthalene lactamide, a compound that contained this methyl structure and an amide configuration, was so readily metabolized by bean plants that it was not possible to recover detectable amounts of the unaltered regulator from plants treated with it. This regulator could not be detected in the main veins and petioles of leaves treated only 24 hours earlier with the compound. The main veins of leaves to which it was applied did contain, however, one detectable reaction product. Two additional reaction products were detected in the petioles of these treated leaves and still a fourth metabolite was recovered from the stems of the plants. This metabolism of the naphthalene was associated with a greater growth response than occurred when an equal molar amount of naphthaleneacetic acid was applied (Mitchell, Linder and

Smale 1960). The ability of roots to exude leaf-applied alpha-methoxyphenylacetic acid and some other methoxy-substituted regulators in what appears to be an unchanged form would seem, on the other hand, to depend on the inability of plants to metabolize this compound readily (Mitchell, Smale and Preston 1959). It is possible that the stability of these regulators within plants is related to their exudation by roots in relatively large amounts.

POSSIBLE DEVELOPMENTS IN THE FUTURE. In view of the large number of physiological responses already obtained with regulators, it is reasonable that many more important developments will result from research with these chemicals. For example, a more thorough and basic understanding of chemical retardants may lead to practical methods of increasing the resistance of fruit trees to low temperature. Preliminary steps in this direction have already been taken. Hendershott (1960) reported that maleic hydrazide showed promise when used experimentally to prolong the dormancy of young citrus trees so that they could better withstand low temperatures. There is some evidence that growth retardant chemicals may also increase the ability of plants to withstand drought (Teubner and O'Keefe 1960). It may be possible to control with chemicals the size and shape of some kinds of plants and in this way facilitate the use of machines to harvest these crops. Although we are able to chemically accelerate ripening of fruits, there is need for a chemical means of retarding to some extent their rate of ripening.

There is evidence that the storage life of some leafy vegetables such as lettuce can be lengthened through the use of regulating chemicals to retard senescence (Bessey 1959). Based on current information, some regulating compounds may be useful in reducing crop losses due to some kinds of nematode and fungus diseases (Davis and Dimond 1953, Marth and Mitchell 1952, Stewart and Leonard 1960 and Walter, Austenson and Courtney 1960). Some chemicals have shown promise when used as gametocides to aid in development of plants with hybrid vigor (Eaton 1957), but our knowledge of how to use regulators for flower initiation and how to control various steps in the reproduction of plants is very limited.

With these challenging needs facing us and with numerous regulators and plant responses yet undiscovered, the future of chemical growth control for plants is, indeed, bright.

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Alteration of plant growth by chemicals

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To review all the chemicals which effect the growth of plants is a herculean task and even when writing books on the subject, as we know it today, one would inadvertently leave out or slight whole portions of the work. Therefore, in a short talk I really cannot detail anything on this subject. I presume that I am participating in this program because of the recent synthesis and physiological studies of a series of chemicals which cause plants to grow with shorter stems than normal, and which are also mutually antagonistic in action with the gibberellins. With that presumption I shall examine the subject, "Alteration of Plant Growth by Chemicals", by speculating on the control of plant growth through the action of several substances in unison, and I shall detail the results only of our own work and to some extent the gibberellin story.

Plant growth may be regulated by quantitative variations in the amount of growth substances at the affected site. This concept has dominated our considerations because of the classical experiments which showed that the extent of an auxin effect (i.e., curvature or apical dominance) was dependent upon the amount of IAA present.

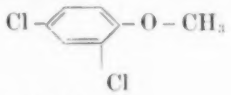
In searching for new growth substances, the consideration of a second regulatory principle is helpful, namely, a check and balance system with two opposite types of growth substances for each effect: one to stimulate and one to antagonize. Chemical compounds are known which may be classified as anti-auxin and anti-gibberellin on the basis of the type of plant growth which is induced by these chemicals. These compounds may not be enzymatic antagonists in a true chemical and biochemical sense and thus the term anti-auxin is unfortunate. Rather, each group of compounds may be growth substances by definition, although the ultimate growth response which they induce may be described as the opposite from that produced by another class of compounds. Thus, the action of two groups of compounds with opposite effects will result in a nearly normal overall development of the plant. I would like to have you bear in mind these two hypotheses in considering the various chemical growth substances.

AUXIN AND ANTI-AUXINS: Much work has been in progress since 1935 with the chemical structures of synthetic auxins as related to biological

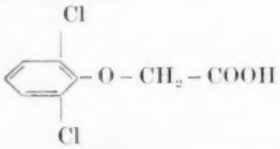
growth response. Audus (1959) repeats that, in general, active molecules can be predicted within chemical limits. Exceptions to most of these limits are known, but the limits are often valid for determining the most active structures. Compounds with auxin activity must have: (1) a ring system as a nucleus; (2) at least one double bond in this ring; (3) a side chain possessing a carboxyl group or a group easily convertible into a carboxyl group; (4) at least one carbon atom between the ring and the carboxyl group; and (5) a particular spatial relationship between the ring system and the carboxyl group. The nature and degree of substitutions on the nucleus and side chain affect activity. Almost all of the hundreds of auxin molecules that have activity are man-made, although IAA is a naturally occurring auxin.

Besides IAA, there are many "auxin"-like substances that have been extracted from plants and detected by chromatographic means (Steward and Shantz 1959, Bentley 1958). Although these compounds are not all auxins or indole compounds by chemical structure, they have auxin-like activity. Thus, IAA does not seem to be the only chemical in plants that has auxin activity, and it is, in fact, not detectable in many plant tissues (Bentley, 1958).

An anti-auxin by definition inhibits growth by counteracting auxin action. Strictly speaking, the term anti-auxin should be reserved for those compounds which compete with IAA for some specific reaction center in the growing cell. Such compounds as, 2,4-dichloroanisole,



and 2,6 dichloropehnoxy acetic acid,



seem to fit the chemical and physiological concept of an anti-auxin. None of them so far have been found to be naturally occurring. On the other hand, naturally occurring compounds, such as the coumarins have biological anti-auxin activity, but not a chemical structure similar to indole acetic acid. Thus, in the auxin field, there are examples of compounds which appear to have the structure and physiological properties of anti-auxins. In addition there are compounds of dissimilar chemical structure which have anti-auxin effects on the plants. The naturally occurring physiological anti-auxins may not even effect the same enzymatic site as that attacked

by IAA. One area for extensive future development in auxinology will be the isolation and identification of the natural auxins and anti-auxins.

HERBICIDES: Chemical treatment of plants must include a mention of the many herbicides. In a review in 1958, Woodford, Holly and McCready described the formulae of some of the many newer chemical compounds which are classified as herbicides. These structures include the chloro- and methyl-substituted acetic acids, the substituted amides such as maleic hydrazide (Brian and Hemming 1957), thioamides and amidine groups such as amino triazole. These multitudes of compounds represent a few specific basic structures which have been extended by synthesis of many homologous compounds. The most active derivatives are promoted by industry, and in the past few years this area of agricultural chemistry has been almost exclusively devoted to synthesis of these herbicides for the eradication of susceptible plants. Some of these chemicals affect plants in interesting ways, but little has been done concerning the action of these substances on plants at levels of application less than lethal amounts. Probably all of them are anti-metabolites and not hormones.

KINETIN: The hormonal effect from kinetin, another class of growth substances, on stimulating cell division and its structural relationship to adenosine suggests that when we know how kinetin acts, we may find that it fits the concept of an anti-metabolite in nucleic acid metabolism. So far it has not been reported as a naturally occurring plant growth substance.

GIBBERELLINS: Gibberellins should now be regarded as a class of naturally occurring plant growth hormones, isolated from plants and effective on plants (Brian et al. 1960, Phinney and West 1960). The primary action of gibberellin is upon stem elongation. Gibberellins induce responses similar to those normally induced by long-day photoperiods or by vernalization. The stem elongation is a consequence both of increased cell multiplication and of an increase in cell size. In the past few years considerable work has been done with these substances by many plant investigators. This avalanche of papers has been based upon the chemical investigations from only a couple of laboratories. Even today only several chemists are studying gibberellins. What would happen to the gibberellin literature if there were 10 times as many chemists investigating the gibberellins?

The isolation of natural gibberellins is a current area of research. The two latest reviews on the natural occurrence of gibberellins were published this year by Brian, Grove and MacMillan (1960) and by Phinney and West (1960). These papers were out of date by publication time due to information contained in papers at the fall 1960 ACS symposium and particularly to the work in England which was presented by MacMillan at that symposium on gibberellins. This group described in detail the chemical structure and probable stereo configurations of nine gibberellins (Brian et al. 1960, Cross et al. 1960a, b, MacMillan et al. 1960). A_1, A_2, A_3,

A_4, A_7, and A_9 have been isolated from fungus. A_1, A_5, A_6, and A_8 have been isolated from unripe bean seeds, *Phaseolus multiflorus*. All of these gibberellins have biological activity on plants. Gibberellin A_1 is, so far, the only one that has been isolated from both plants and fungi. This difficult isolation work needs to be repeated for many other plants, but the motivation may not be great enough to stimulate the effort which is involved. All of the nine gibberellins are effective on plant growth, but each gibberellin affects certain plants more than others. This type of result suggests that the various plants contain different gibberellins. Since gibberellins A_6 and A_9 are not available yet, it is too early to know much about the action spectra of the various gibberellin structures on the various species and varieties. How many of these gibberellin structures will eventually be isolated as natural plant components is also only a matter of conjecture at this time. How many structures the chemist may make in addition is still a matter of another order of magnitude. The chemist may introduce halogens or other substituents into the carbon rings; they may prepare stereo isomers (and there is an unusually large number of asymmetric carbons in a gibberellin structure); they may make esters or glucosides with the hydroxyl groups; and they have already made active ester derivatives of the carboxyl group. These synthetic derivatives may have both gibberellin and anti-gibberellin activity; both would be of economic and theoretical value. Little or no work is being done on this synthetic approach now.

Studies on the biogenesis of the gibberellins have begun by labeling the gibberellins with C^{14} acetate and 2-C^{14} mevalonic lactone (Brian et al. 1960). Without giving the details, their data suggest that a tricyclic diterpene skeleton may be a probable intermediate which rearranges to give the diterpene structure with a bridge between the 2 rings. For comparative biochemistry it is interesting to note the similarities in precursors and the probable intermediates in gibberellin synthesis and in the synthesis of the many complex terpenes by plants. This leads to the speculation of whether the naturally occurring terpenes have any gibberellin or anti-gibberellin activity.

The enzymic interconversions among the gibberellins will also be an area of future research. Interconversion of the gibberellins by enzymes could be a growth controlling mechanism dictated by the genetic control of the enzyme needed for these changes. Such interconversions among the gibberellins, which have different growth responses for each plant, would permit a great latitude for controllig growth even without anti-gibberellins.

ANTI-GIBBERELLINS: One interpretation of the mode of action of the gibberellins has been that they block the action of some inhibitory influence in the intact plant, and, as a result, release the full growth-promoting influence of the endogenous auxin, IAA (Audus 1959). A somewhat

similar hypothesis is that there may be growth inhibitory substances in plants which are anti-gibberellins. Even if there should not be any naturally-occurring anti-gibberellin, chemicals of synthetic origin which would modify plant growth in an opposite way from the gibberellins, may be of great value to mankind for the control of plant growth.

I became involved in this particular problem from a basic research program concerning our discovery that as much as 30 per cent of the total soluble phosphorus in a plant root and sometimes in the leaves, was present as phosphorylcholine, $(CH_3)_3N^+–CH_2–CH_2–O–PO_3H^-$. In an effort to block the action of this compound in the plant, a chlorine atom was substituted for the OH group of choline so that the new molecule could not be esterfied by the phosphate group. This structure, $(CH_3)_3N^+–CH_2–CH_2Cl \cdot Cl^-$, (2-chloroethyl) trimethylammonium chloride, is an analog of choline in which the alcohol group has been replaced by a chlorine atom. Thus, its trivial name is chlorocholine chloride which has been abbreviated CCC. We have prepared and tested about 100 derivatives of this general structure, $(CH_3)_3N^+–CH_2–CH_2X \cdot Cl^-$ (Tolbert 1960a, b, Wittwer and Tolbert 1960a, b, Lindstrom and Tolbert 1960). As analogs of choline, they have some choline esterase activity. All the active derivatives would fit a hypothetical enzymatic site in the plant of the same structural characteristic as choline esterase, an enzyme not known to occur in plants. This site has a point of attachment for the ammonium cation of the chemical. The ammonium cation must have 3 methyl groups for biological activity on plants. A second position of the effected site is specific for the X constituent of the molecule. For optimum activity the X substituent in the compound must be the length of a 2 carbon chain from the ammonium cation. In active compounds, substituents at X were of small size, nucleophilic and nonionizable. The most active derivatives were the chloride or bromide salts of $(CH_3)_3N^+–CH_2–CH_2Cl$, $(CH_3)_3N^+–CH_2–CH_2Br$, $(CH_3)_3N^+–CH_2–CH=CH_2$, $(CH_3)_3N^+$

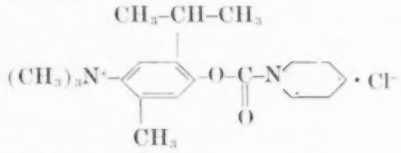
$CH_2CCl=CH_2$ and $(CH_3)_3N^+–CH_2–CH–CH_2$. Other active derivatives were $(CH_3)_3N^+–CH_2–CH_2–O–CH_3$, $(CH_3)_3N^+–CH_2–CH_2–S–CH_3$, $(CH_3)_3N^+–CH_2–CHF_2$, $(CH_3)_3N^+–CH_2–CH_2–CH_3$ and $(CH_3)_3N^+–CH_2–CH_2SH$. Why such compounds alter plant growth is unknown.

The most characteristic growth alterations produced by CCC derivatives were shorter plants with short internodes, thick stems and dark green leaves. CCC derivatives inhibit seed germination and tend to duplicate the effect of short photoperiods and exposure to low temperature. This class of compounds has been considered by us as a new growth substance, for these growth alterations can be obtained with sensitive plants without

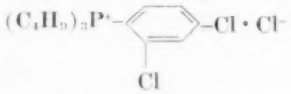
change in wet or dry weight. They are not, therefore, inhibitors or retardants if properly applied. In fact, very low concentrations of CCC stimulated the growth of some plants.

In general, the growth response produced by CCC appeared the opposite from that induced by gibberellin and a mutually antagonistic effect can be obtained from simultaneous application of both a CCC compound and gibberellin. The details of these results suggest that CCC was altering plant growth in a manner which may be described by the term anti-gibberellin. However, the chemical structure of gibberellin and CCC in no way resemble each other.

CCC is not unique in its ability to cause plants to grow shorter and thicker stems. AMO 1618,



is effective for producing chrysanthemum plants and cucurbits with short stems (Krewson et al. 1959, Cathey 1959). Phosfon D,



is also effective for producing short stemmed chrysanthemums. CCC influences a much broader spectrum of plant species than phosfon D or AMO 1618 without being toxic. CCC is relatively cheap and it is structurally related to a known metabolite of plants, namely, choline. A fourth type of anti-gibberellin compound is maleic hydrazide (Brian 1957).

Gibberellin in low concentrations can reverse the action of all four of these classes of compounds. These substances vary widely in structure among themselves and in comparison with gibberellin. There does not seem to be any correlations amongst those compounds except in their effect upon the growth of plants. The chemicals are not anti-gibberellins in a biochemical or physiological sense.

NATURALLY OCCURRING INHIBITORS: Many naturally occurring inhibitors from plant extracts have been reported and are obviously very widespread (Bentley 1958). These substances may be anti-auxins or anti-gibberellins or something else. The tedious process of identification of these substances will, in time, sprinkle new knowledge upon plant growth investigations. But often this research has been approached by plant physiologists without sufficient participation with the chemists. When the structure and, if possible, mode of action of many of these new inhibitors

become known, each will move from the category of general inhibitors into that of a specific chemical for inducing a specific growth effect. At present the literature is clouded with reports about these unknown inhibitory substances, and I wish to select only two recent investigations in the field of inhibitory compounds upon plant growth.

The first is a report by Salisbury and Bonner (1960) on the inhibition of photoperiodic induction of the cocklebur by 5-fluorouracil. It is known to inhibit the formation of thymidine and thus it may inhibit DNA synthesis or perhaps nucleic acid metabolism. This example, then, may be a case of a specific compound acting as an anti-metabolite upon a specific synthesis. The details of the mode of action of 5-fluorouracil *in vivo* is in actuality unknown and not simple, but it is an example of the future possible use of chemicals to control and modify plant growth. We do not appear to care what the plant may wonder about this treatment but only whether the residue of the chemical will affect man. 5-Fluorouracil is not a known naturally occurring substance, but that restriction is also certainly not necessary for chemical alteration of plant growth.

For my second example about inhibitory substances in general, I select a case to represent the vague, voluminous literature about the presence of many inhibitory substances from plant parts. There is no better place to go for this example than one's own laboratory. We have been working at the isolation of the substances responsible for seed dormancy during prematurity, maturity and post-harvest dormancy (Miyamoto, et al.). From the seed coat of wheat seeds, we can isolate four different types of chemical substances which are potent inhibitors of seed germination. They are also anti-gibberellin in that they reverse the gibberellin stimulation of seed germination. They are complex, unknown, organic substances of a catechin, catechin-tannin, and alkaloid nature. Since these naturally occurring substances control germination, they too may affect nucleic acid syntheses and be even more active than unnatural inhibitors such as 5-fluorouracil. We cannot help but dream that some day these natural inhibitors will take their place along with natural growth promoters such as indoleacetic acid and gibberellin as known chemicals which control plant development.

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Antimetabolites and plant growth

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One of the largest fields of activity in biological research is the control of growth by the principle of selective toxicity. In this approach, compounds are sought which will modify or inhibit the growth of undesired species, or of tissues such as tumors, with a minimum of adverse effect upon a desired species or the host. Random testing of large collections of chemicals is the usual approach to finding suitable compounds. Sometimes, however, a biologically active substance such as hormone or coenzyme is used as a starting point in the synthesis of analogs. Antimetabolites may be discovered by either of these approaches.

The inhibitory effect of a compound may be discovered by random testing and the fact that it is an antimetabolite may not be found until special studies of its mode of action are carried out.

Antimetabolites, or metabolic antagonists, have been widely used in biochemical research and experimental chemotherapy. A *metabolite* may be defined as a compound that occurs in living organisms and has an essential function in a biochemical process. An *antimetabolite* usually resembles the metabolite closely in its structural formula although this is not always the case. It is recognized by its ability to block the biochemical process carried out by the metabolite. This blocking adversely affects the organism as shown by toxic effects, slow growth or even death. The block may be reversed by adding an excess of the metabolite or by adding the products of the reaction in which the metabolite normally participates.

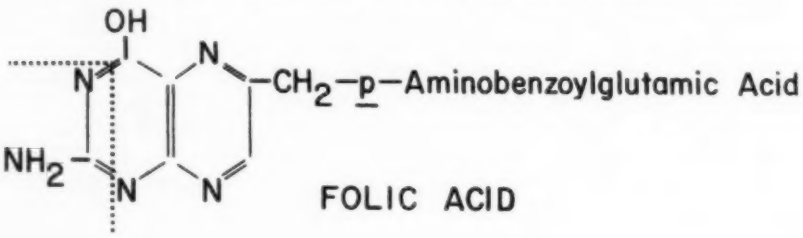
The effect of antimetabolites is related to the mechanism of enzyme action. Enzymes are special proteins that have receptor sites on their surfaces where a specific *metabolite* and a *reacting substance* can be brought in close proximity in an active state so that a chemical reaction takes place. The products of the reaction are then set free from the enzyme surface. An antimetabolite can attach itself to the receptor site on the enzyme because it resembles the *metabolite* so closely or because the antimetabolite has a chemical group that readily combines with the receptor site, and may be said to "fit" this site. However, the antimetabolite may not combine with the *reacting substance*, or it may bind so tightly with the enzyme that the *metabolite* is kept away from he receptor site which is occupied by the antimetabolite which refuses to be displaced. These steps are shown diagrammatically in figure 1.

$$\text{Metabolite (M)} + \text{Enzyme (E)} \longrightarrow \text{ME}$$
$$\text{ME} + \text{Reactant (R)} \longrightarrow \text{MER}$$
$$\text{MER} \longrightarrow \text{Enzyme} + \text{Products}$$

$$\text{Antimetabolite (A)} + \text{Enzyme (E)} \longrightarrow \text{AE}$$
$$\text{AE} + \text{Reactant (R)} \longrightarrow \text{No reaction}$$

Fig. 1 Diagrammatic representation of metabolite-antimetabolite-enzyme relationship.

One of the best examples of the action of an antimetabolite is the relationship between folic acid and the closely related substance, aminopterin. The chemical similarity between these two substances is shown in Figure 2. Folic acid is a vitamin that is present in green leaves and also presumably in all living cells. It is active biologically in the hydrogenated form, tetrahydrofolic acid, which can combine with formic acid or formaldehyde or with other sources of a "single-carbon" unit. The carbon atom thus derived is then transferred to various acceptor substances and during these changes tetrahydrofolic acid may lose two atoms of hydrogen, in which case it must be reduced once again before going through another cycle.



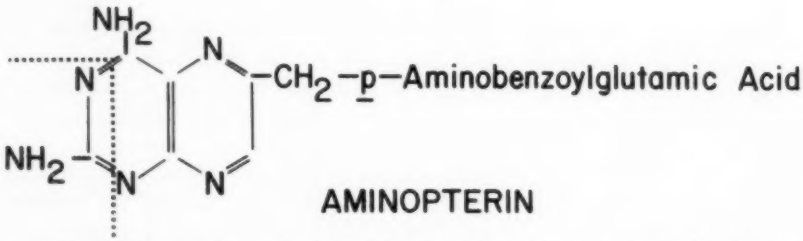


Fig. 2 Abbreviated structural formulas for folic acid and aminopterin, showing the postulated point of attachment to the dihydrofolic reductase enzyme protein.

The addition of aminopterin to many living organisms abruptly halts the action of folic acid and produces markedly toxic effects. For example, the injection of aminopterin at the rate of 2 mg./kg. of body weight in rats produces weight losses, bloody diarrhea, destruction of the lining of the

intestine and liquefaction of the bone marrow (Philips and Thiersch 1949). Plants are also affected, and necrotic changes have been described in the roots, bulbs, and seedlings of onions (Jakowska, Nigrelli and Goldsmith 1949).

We reported some years ago that tetrahydrofolic acid would prevent or reverse the toxic effects of aminopterin in mice while folic acid had a much weaker protective action (Broquist et al. 1951). It was concluded that aminopterin blocked the biological conversion of folic acid to tetrahydrofolic acid. More recently this has been studied with the purified enzyme, dihydrofolic reductase, and some authors have concluded that the toxic effects of aminopterin may be explained on the basis of its action on this one enzyme system.

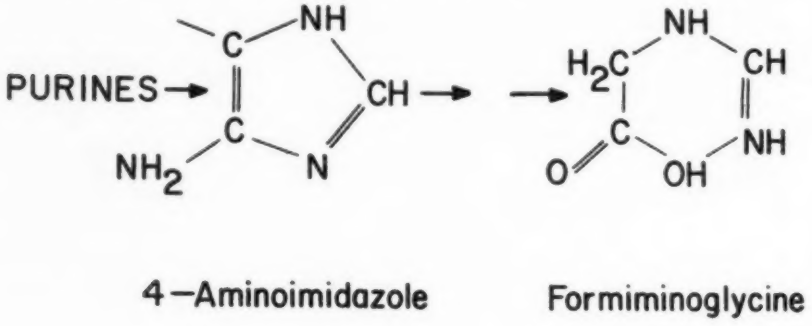
Aminopterin combines with the enzyme dihydrofolic reductase very firmly. Folic acid will not displace aminopterin from combination with dihydrofolic reductase until the concentration of folic acid is 100,000 times as great as that of aminopterin. This has been shown in experiments with an extract of rat liver that was allowed to react with radioactive aminopterin. The enzyme was inactivated and a firm combination took place from which the aminopterin could not be removed by dialysis, and even when folic acid was added at 100 times the concentration of the aminopterin, the activity of the enzyme was not restored. Not until the amount of folic acid was 10,000 times as great as that of aminopterin was the enzyme reactivated to the extent of 85 per cent (Werkheiser 1961). The steps may be summarized by equations similar to those in Fig. 1 as follows:

$$\text{Folic Acid} + \text{Enzyme} \longrightarrow \text{FE}$$
$$\text{FE} + 2\text{TPNH} + 2\text{H}^+ \longrightarrow \text{FH}_4 + \text{E} + 2\text{TPN}^+$$
$$\text{Aminopterin} + \text{Enzyme} \longrightarrow \text{AE}$$
$$\text{AE} + 2\text{TPNH} + 2\text{H}^+ \longrightarrow \text{No reaction}$$

Baker (1959) has suggested that the 2-amino group of folic acid combines with an acidic site on the enzyme. When the 4-hydroxy group of folic acid is changed to an amino group, the 2-amino group becomes a much stronger base so that it is more firmly attached to the enzyme. This would explain Werkheiser's observations.

One of the most intertesting examples of the action of an antimetabolite in plant growth is the weed killer, aminotriazole (3-amino-1,2,4-triazole). This substance is freely soluble in water and is non-irritating to animals. When sprayed on a susceptible plant, no changes are seen at first, but after a few days the leaves turn brown and drop off. The new shoots that appear are white, and evidently the synthesis of chlorophyll has been blocked. Death of the terminal meristem occurs, followed by a gradual dieback of the stem and roots. Aminotriazole is used widely as a weedkiller for Canada thistle, quack grass, poison ivy, aquatic weeds and a number of other leafy and woody species (Am. Cyanamid Co. 1957).

The action of aminotriazole as an antimetabolite was studied by Rabinowitz and Pricer (1956) who described the bacterial breakdown of purines by the organism *Clostridium cylindrosporum*. It was possible to separate the various steps in this breakdown and it was found that xanthine was broken down to 4-aminoimidazole, which is quite similar in chemical structure to 3-amino-1,2,4-triazole, fig. 3. The ring of 4-aminoimidazole was then found to be broken down further to produce formiminoglycine.



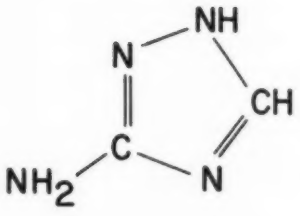


Fig. 3 Purine degradation by Clostridium cylindrosporum to show the step that is blocked by aminotriazole.

Aminotriazole was an active inhibitor of this enzymatic step and one equivalent of aminotriazole was sufficient to block the breakdown of about 20 equivalents of 4-aminoimidazole. These observations at first did not appear to be related to the effect of aminotriazole as a weed killer. However, it was

also found that aminotriazole would inhibit the growth of certain microorganisms including *Escherichia coli* and *Torula cremoris* (Weyter and Broquist 1960), and *Saccharomyces cerevisiae* (Hilton 1960). The inhibition was reversed by adenine, histidine or a combination of the two. Both of these compounds contain the imidazole ring. This made it appear that aminotriazole could block the synthesis of the imidazole ring as well as its breakdown.

Studies with green plants were described in which it was found that the bleaching effect of aminotriazole could be reversed by treating the leaves with a solution containing adenine and histidine. It was also found that riboflavin would reverse the effect (Sund, Putula and Little 1960). Aminotriazole was found to inhibit the synthesis of riboflavin by the mold *Eremothecium ashbyii* which is used for the commercial production of riboflavin (Sund and Little 1960). This biological synthesis has been shown to take place from a purine precursor (Brown, Goodwin and Jones 1958, fig. 4), so that aminotriazole has a blocking effect on the formation

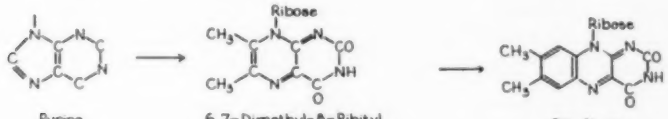


Fig. 4 Steps in the biosynthesis of riboflavin by *Eremothecium ashbyii*.

of several metabolites that contain an imidazole ring or are derived from a substance containing an imidazole ring. Furthermore, in the biological synthesis of riboflavin, it has been reported that the imidazole ring of adenine opens and a pyrazine ring is then formed (McNutt and Forrest 1958). Perhaps this is the step at which aminotriazole blocks riboflavin synthesis, since it is known that aminotriazole blocks the opening of the imidazole ring in the degradation of xanthine.

In the photosynthetic microorganism *Ochromonas danica* it was found by Aaronson (1960) that the inhibitory action of aminotriazole on growth and the synthesis of chlorophyll and carotene was reversed by ferrous and ferric ions. However, iron did not reverse the growth inhibition caused by aminotriazole in *O. malhamensis* and *Euglena gracilis*

The action of aminotriazole on animals appears quite different from its effect on plants. In animals, the substance has the property of inhibiting the formation of thyroid hormone by preventing or "blocking" the thyroid gland from combining with iodides (Alexander 1959). A number of other ring compounds have this "antithyroid" property, and many plants, especially in the *Cruciferae*, contain such antithyroid compounds (Greer

and Astwood 1948). The prolonged administration of an antithyroid compound to rats at thyroid blocking levels produces a compensatory overproduction of the thyroid-stimulating hormone of the pituitary gland which is normally kept in check by the presence of thyroid hormone in the bloodstream. The result after a long time on this regime is that changes develop in the rat thyroid gland, and eventually these changes take on the appearance of tumors. Work is in progress in our laboratories on the effect of adding thyroid hormone to the diet of rats receiving aminotriazole. It is anticipated that the thyroid glands of these rats will not develop the the changes that were described in a recent publication as being associated with the feeding of aminotriazole (Jukes and Shaffer 1960).

There is a most interesting similarity between aminotriazole and the chemotherapeutic substance sulfanilamide. Both substances are antimetabolites which are selectively toxic for members of the plant kingdom and not for animals. Both block the synthesis of vitamins, in one case that of riboflavin and in the other case that of folic acid. These two vitamins are synthesized by plants and not by animals, so that animals must obtain the ready-made vitamins from their diet and hence are not harmed by an antimetabolite that interferes with the biological synthesis of the vitamin. Interestingly enough, both aminotriazole and sulfanilamide are antithyroid substances.

There are other examples of antimetabolites in plant growth, for example dichloropropionic acid appears to block pantothenic acid (Hilton, Jansen and Gentner 1958). The study of antimetabolites and their effects has not been as rapid a method of obtaining useful compounds as have more empirical approaches such as random testing, but an understanding of metabolic processes in plants may be greatly aided by discoveries in the field of antimetabolites.

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Test-tube studies on flowering: Experiments with the Lemnaceae, or duckweeds.[1]

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The title is, of course, a deliberate eye-catcher, but it also describes an important goal of this work—the study of flowering under completely controlled conditions. Ever since the discovery of photoperiodism, investigations of flowering have been widely conducted, and it is obvious, for example from Dr. Borthwick's paper, that the field has progressed impressively. It has occasionally been handicapped by the fact that the usual experimental plants grow and flower relatively slowly, and, being large, are not easily kept under controlled conditions. For this reason, the Lemnaceae (duckweeds)—the smallest known flowering plants—have frequently been suggested as experimental material for such work. Although research with these plants has actually been undertaken by few investigators, it may already have contributed some useful new insights into the photoperiodic control of flowering. Only further results will tell whether it has or not, but the history and present status of the work is interesting in itself, and worth summarizing.

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The Lemnaceae are monocotyledons, often regarded as extreme reductions of the Aralean line. They are all fresh-water plants that float at or just below the surface of the water. The individual green bodies are referred to as "fronds" to avoid committment as to their actual nature, since they probably represent stem, leaf and bud tissues. They are always less than a centimeter long, commonly a few millimeters. In the genus *Lemna* they are thin and flat, approximately ovoid in outline, and bear a single root on the lower surface. New ("daughter") fronds arise vegetatively from meristems enclosed in two pockets lying on either side of the more pointed end of the "mother" frond, and the daughters become mothers in their turn. Daughters remain attached to their mothers for some time after reaching full size—even occasionally after producing daughters themselves—so that the commonest growth form is that of "colonies" in which several fronds appear together. When flowering occurs, any given frond bears a single flower in one of the two meristematic pockets. The flower consists of a pistil and two stamens surrounded by a membranous "spathe", and on fertilization gives rise to a one- or few-seeded utricle.

The salient advantages of *Lemna* and its relatives as experimental organisms are easily stated. Once a single frond has been made aseptic in any of a number of ways, it will give rise to a clone that may be maintained indefinitely under aseptic conditions by standard microbiological techniques. Hence, the effects of chemical agents can be studied without interference by micro-organisms. The use of the same clone or clones throughout an investigation eliminates genetic variation as a factor. Growth is rapid, at rates depending on the particular species and conditions used. Commonly, a culture started with a single 3-frond colony will contain 40 to 80 fronds after a week or ten days. The small frond size makes it indeed possible to grow such a culture with 15 ml of medium in a test-tube, justifying the title; however, 20–50 ml of medium in a small flask is the more usual culture condition. Thus, the amount of space and time required for complex experiments is greatly reduced.

For more detailed information on all the above topics see the writer's review (Hillman, 1961c); only observations on flowering will be considered from here on.

One reason for interest in the flowering of Lemnaceae has been its apparent rarity in nature. This reputation for rarity has probably been due in part to the inconspicuous nature of the flower, but it seems certain that most species flower irregularly. Saeger (1929), perhaps the first investigator to urge the study of flowering in these plants, reviewed the older literature, and concluded from his own observations that the chemical composition of the pond water played an important part in controlling flowering. For example, in a particular pond near Columbia, Mo., both *Spirodela polyrrhiza*

and *Wolffia papulifera* were found in flower, while at the same time, in many other ponds within a radius of twenty miles, these species were entirely vegetative. Landolt (1957) made extensive field observations in California, where at least four species were found in flower. However, only a few strains (clones) of three of these species would flower under controlled conditions (see below). Such observations might make one expect particularly interesting mechanisms in the flowering of these plants.

The first published experiments on Lemnaceae flowering are those of Hicks (1932) conducted in crude culture. Five species of *Lemna* and one of *Wolffia* flowered following treatments with ultraviolet radiation, and three of the *Lemna* species also flowered following vegetatively injurious treatments with sodium hydroxide. As Kandeler (1955) points out, Hicks made no attempt to see whether flower primordia were already present in these plants, but the paper states clearly that untreated controls failed to flower. No confirmations or extensions of this work have been reported, so its status at present remains uncertain.

The first unequivocal success in controlling flowering in the Lemnaceae was obtained by Kandeler (1955, 1956) with several strains of *Lemna gibba* in aseptic culture. In these experiments, flowering was evaluated quantitatively by assigning arbitary values to the various developmental stages of flower primordia observed by dissection, and was found to depend on at least three factors: light quality, photoperiod, and the "age" of the culture medium.

If artificial light containing a considerable proportion of far-red radiation (supplied by incandescent light) was used, Kandeler found that his *L. gibba* respond as a long-day plant, with a critical daylength of 12 to 14 hours. In addition, far-red radiation appeared to be more effective than red in promoting flowering when used to interrupt long nights. It is surprising—although perhaps only in hindsight—that Kandeler devoted most of his investigation to establishing crude action spectra—work of a kind done with so many plants—while virtually ignoring his own most interesting observation. This was that, under fluorescent light (low in far-red radiation), flowering failed to occur irrespective of photoperiod if the plants were frequently transferred to fresh mineral medium. Under those light conditions, flowering occurred (under long days) only in medium "aged" by the growth of the plants—that is, if the plants were allowed to remain in the same medium for some time. As will become evident, this result appears to be directly connected with others suggesting a new approach to the photoperiodic control of flowering. However, Kandeler pursued the "aged" medium effect no further, but simply eliminated it from his experiments by making frequent transfers.

The next observations of Lemnaceae flowering under controlled, aseptic

conditions were those of Landolt (1957). Several strains of *L. gibba*, *L. minor* and *L. perpusilla* flowered under circumstances suggesting that relatively high temperatures and light intensities, and crowded conditions in the cultures, were the promoting factors. Because these observations were more or less incidental to a survey of the growth of many species and strains under various light and temperature combinations no attempt was made to study them in greater detail. Probably the most important general lesson to be learned from Landolt's work is that different strains of the same species may differ markedly with respect to both flowering and vegetative growth requirements.

Most of the remaining work to be discussed is that of the writer, either published or in press (Hillman 1958, 1959a, b, 1960, 1961a, b). A few hitherto unpublished observations will also be mentioned. This work has been carried out largely with strains kindly supplied by Dr. Landolt and Dr. Kandeler, mentioned above.

The start of the author's work was from further observations on Landolt's *L. perpusilla*, strain 6746. As Landolt himself had found, it flowers under all photoperiods in old, crowded cultures. However, under short-day conditions (8 hours of light per day) it flowers also in fresh cultures, producing visible flowers within 10 days, at which time not even flower primordia are detectable under 16 hour photoperiods. Cultures can be maintained vegetative indefinitely under 16 hours of light per day if transferred to fresh medium every two or three weeks. The flower-promoting effect of short days can be completely abolished by brief interruptions of the long night periods with dim red light; thus, in fresh medium, this plant responds as a typical short-day plant (Hillman 1958).

Further experiments on this short-day response were undertaken, using as a quantitative measure of flowering the percentage of fronds in a culture bearing flower primordia at any given time (abbreviated FL%). Experiments generally ran from 6 to 10 days from the start of the cultures. Maximum FL% values were obtained with 6–11 hours of light per day, and even under total darkness if sucrose was supplied; no flowering occurred with daylengths longer than about 15 hours. A single long night proved sufficient to induce a measurable degree of flowering in cultures subsequently placed in long days. Attempts to reverse the flower-inhibiting effect of red light night-interruptions with far-red radiation failed, for reasons to be mentioned later (Hillman 1959a).

The succeeding paper (Hillman 1959b) turned to the effects of medium composition. Flowering even under long days in crowded cultures suggested that sufficient growth had caused some change favorable to flowering in the composition of the medium. The reader will be reminded of Kandeler's observations, but should beware of two differences. In the first place, in Kandeler's *L. gibba* work, the "aged" medium conferred sensitivity to

photoperiod under conditions in which it was not otherwise present—that is, "aged" medium allowed a long-day response. In *L. perpusilla* 6746, the "crowded" medium conferred insensitivity to photoperiod—that is allowed flowering under all daylengths. Secondly, the medium (Hutner's) used by Landolt, and thus at first by the writer (1958, 1959a) was quite unlike that used by Kandeler, containing a high level of the chelating agent ethylenediaminetetracetic acid (EDTA) and of many trace metals. Hence, it would have been an error, as subsequent results showed, to equate Kandeler's "aged" with Landolt's "crowded" medium.

Preliminary experiments were done to distinguish between the possibilities that "crowded" medium was rendered favorable to flowering for *L. perpusilla* 6746 either by (a) the excretion of some material by the plants or (b) the removal of some material by them. The second, rather than the first possibility was indicated by the observation that flowering under long days took place much more rapidly in dilute rather than in full-strength Hutner's medium, although the growth rates were not much different. Thus, the next step seemed to be the "dissection" of the medium to determine what components might be involved. Because this would be a complex procedure with a highly chelated medium such as Hutner's, another medium (Gorham's) was tried, of the Hoagland's type, containing only a low level of tartaric acid to maintain iron availability. This was much more like the medium used by Kandeler for *L. gibba*, although not identical with it.

This choice of another medium proved lucky, though for a completely unforeseen reason. It immediately became obvious that in Gorham's medium *L. perpusilla* 6746 is completely daylength-indifferent, flowering rapidly under all photoperiods even in fresh, uncrowded cultures. Why then the typical short-day response in Hutner's medium? Though there are several differences between Gorham's and Hutner's, the most obvious is the presence of EDTA in the latter, and this proved to be the operative factor. With EDTA added to Gorham's medium at levels of $1–3 \times 10^{-5}$ M, flowering is completely or substantially inhibited under 16 or 24 hour photoperiods, but is unaffected under 8 hour photoperiods. In short, EDTA turns *L. perpusilla* 6746 from a daylength-indifferent into a short-day plant. EDTA is not the only compound with this effect; other chelating agents, not only of the nitriloacetic acid type (like EDTA) but also including citric and tartaric acids, have the same action, though the effective concentrations vary.

Also included in the same paper (Hillman 1959b) were data on the effects of temperature. The results described above hold at temperatures in the range 22°–27° C. At about 31°C., in the presence of sucrose, *L. perpusilla* 6746 grows well but fails to flower irrespective of medium or photoperiod. In a narrow intermediate temperature range (Ca 29.5°) which can

be accurately maintained by immersing the culture tubes in a thermostated water bath, a short-day requirement is observed irrespective of medium. Thus, at this temperature, high levels of chelating agents are not required to bring about a short-day response—that is, inhibition of flowering in long but not in short days.

The action of chelating agents on *L. perpusilla* 6746 suggested that metal metabolism might play an important role in photoperiodism, a notion by no means original with the writer. However, alternative interpretations are quite possible from these results alone—the simplest alternative would be that the chelating agents merely acted as more or less specific flowering inhibitors, but that the "flowering impulse" was stronger in short days. That this explanation would not be entirely adequate, however, is suggested by the action of gibberellic acid. This substance promotes vegetative growth but inhibits flowering about equally in both long and short days (Hillman 1960). Nevertheless, it seemed desirable next to see what effect chelating agents might have on *L. gibba*.

In preliminary experiments, Kandeler's strain G3 of *L. gibba* grown in a modified Gorham's medium (designated M), under continuous fluorescent light, flowered if the medium was allowed to "age" but not if it was changed frequently. These results confirmed Kandeler's observations, done originally with his strain G1. Although attempts to confirm Kandeler's report on the effects of light quality have not been successful, numerous details of these experiments, including light sources, differed from those of Kandeler's, and this aspect of the problem will not be pursued here. The major result of interest was that EDTA, instead of inhibiting flowering in *L. gibba* G3, entirely substituted for "aged" medium; in M medium with EDTA, flowering takes place rapidly in long days whether or not the medium is changed frequently (1961a).

A detailed comparison between the activities of chelating agents (mainly EDTA) and M medium "aged" by *L. gibba* G3 was then undertaken, with the following results. EDTA promotes rapid flowering of *L. gibba* G3 under long days. In short days the plant never flowers. Tartaric acid acts like EDTA; thus, both agents mimic "aged" medium. To compare the action of EDTA on *L. gibba* G3 with that on *L. perpusilla* 6746, the relation of pH to activity was studied. EDTA in both systems is more active at about pH 5 than at pH 4, although in the first case its effect is to promote flowering in long days and in the second to prevent it. Two further similarities between the effects of EDTA and "aged" medium were then established. First, "aged" medium inhibits the flowering of *L. perpusilla* 6746 in long days but not in short. Second, the activity of "aged" medium, like that of EDTA, is greater at the higher pH level.

It was also observed that the production of "aged" medium depended on the growth of *L. gibba* G3 plants in it, but not necessarily under long days. Medium "aged" by growth under 8-hour photoperiods, where there

is no flowering, is still effective (Hillman 1961b).

An extensive discussion of these results cannot be given here, but certain conclusions may be suggested. A minor point concerns the nature of "aged" medium. The simplest hypothesis is that it contains a substance or substances—organic or amino acids, perhaps—excreted by the plants during growth and active as chelating agents. One might set out to identify these substances, but the fact that their production is clearly not photoperiod-specific, and that their action is mimicked by EDTA, giving a system more easily studied, makes such work unrewarding with regard to the major points of interest. The latter can be briefly summarized:

EDTA or "aged" medium cause or promote flowering in *L. gibba* G3 under the same general circumstances in which they inhibit it in *L. perpusilla* 6746; that is, in long but not in short days. The conclusion that these agents affect the response to photoperiod rather than merely flowering is almost inescapable. In their absence, *L. perpusilla* 6746 is daylength-indifferent, and *L. gibba* G3 fails to flower. In their presence, the one becomes a short day plant and the other a long day plant. Thus, these agents can best be regarded as conferring sensitivity to long photoperiods, with results coupled to flowering in opposite ways in the two plants.

If it is at least tentatively accepted that chelating agents are thus modifying photoperiodic sensitivity, or conferring it, the mechanism becomes of the greatest interest. The most reasonable hypothesis is that they act on metal metabolism, perhaps some aspect of trace metal function. Many attempts have been made to reverse these effects by adding various ions to the medium, but none have been successful. It is not even clear whether the action is primarily in the medium or within the tissues themselves (see Hillman 1961a, 1961b). It has been possible to show that photoperiodic induction in *L. perpusilla* 6746 is far more sensitive to iron deficiency than is vegetative growth (Hillman 1961a) but this in itself is no evidence that iron metabolism is the area in which the chelating agents act. However, some recent, unpublished results, which must be accepted with caution at present, may provide a system in which the action of chelating agents can be more accurately identified.

One might hope to distinguish between action in the medium and action within the plant by assaying the effectiveness of EDTA in various dilutions of the basic M (Gorham's-type) medium. Either of two results was expected from such assays. EDTA might be more effective in dilute medium, thus indicating action by reducing uptake of some component of the medium. Or, the effectiveness of EDTA might be relatively independent of medium dilution, reflecting action within the tissue only. However, the actual results, so far, have consistently embodied a third, unexpected possibility: on both *L. perpusilla* 6746 and *L. gibba* G3, EDTA is *less* effective in dilute ($\frac{1}{3}$–$\frac{1}{5}$) than in full-strength medium. This result is of interest if only because it shows the first procedure by which the effectiveness of

EDTA in these systems has been successfully reduced—other than low pH, of course, and pH change has been excluded as a variable in these experiments. Such data also suggest that EDTA interacts with some component in the medium to bring about its effect, possibly *increasing* the uptake of that component. Most important, they provide a system in which to test for that component, although such experiments are subject to considerable quantitative variation, and other pitfalls may arise. However, it may soon be possible to say what substance, presumably a metal, is apparently so closely linked to photoperiodic sensitivity.

Several loose ends may be mentioned here. The results with *L. gibba* G3 make it clear that EDTA added to M medium, like "aged" medium, confers photoperiodic sensitivity, with resultant flowering in long days. It is, therefore, remarkable that in Hutner's medium under the same conditions flowering is absent or extremely low. For *L. gibba* G3 then, unlike *L. perpusilla* 6746, Hutner's medium is not equivalent to M with EDTA. Another but related group of observations deals with other strains of the two species used here. Some, but not all of the *L. gibba* strains tested respond like *L. gibba* G3. Comparative studies with several other strains of *L. perpusilla* (all of these are from Landolt) and with a strain of *L. paucicostata* (perhaps synonymous with *L. perpusilla*—see Hillman 1961c) have given the following tentative results. One fails to flower under any condition used; another responds as does 6746, but more slowly; a third flowers under all daylengths in M or M with EDTA, but not at all in Hutner's; the *L. paucicostata* seems to be a short-day plant irrespective of medium. Thus, there is abundant material for further work, and some of the results should bear on the problem of chelating agents, metals and photoperiodism. More work, of course, might also be done on the responses of all of these to temperature and light quality as well, for a completely comparative approach.

An investigation by Purves (1961) explains another disconcerting observation mentioned earlier—the failure of far-red radiation to reverse the effects of red light night interruptions on *L. perpusilla* 6746. It now appears that this plant, in chelated media, responds like the seedlings of the short day Japanese morning glory (*Pharbitis*). That is, the red, far-red system is present and active, but not in the most usual way. Far-red given at the start of the dark period (or in it) *inhibits* induction; the effect at the start of the dark period is reversible by red. This result is of particular importance since the ineffectiveness of the red, far-red system on flowering that might have been inferred from the earlier results might help fortify the doubts, occasionally expressed, whether an understanding of flowering in such unusual plants as the duckweeds will have any relevance to general mechanisms in other plants. Dr. Purves has now removed one basis for such doubts. As to the general question, no final answer can be given. It is the writer's opinion that the major difference between

the Lemnaceae and other plants in this type of work is the ease with which applied substances can reach the entire plant, due to the morphological situation. This may be the reason the effects of chelating agents are so easily demonstrable; they may, of course, turn out to be equally so with certain other plants of the more usual structure. Whether they are or not, if the underlying mechanisms are as basic as they appear to be, they will eventually prove relevant elsewhere.

Since by now the major points of this small history may have been completely obscured, a brief summary is in order. The duckweeds (Lemnaceae) possess several unique advantages as experimental organisms for the study of flowering. In nature, their flowering is usually irregular and is, perhaps, controlled in part by the chemical composition of the water. Work by several investigators including the writer has led to the discovery that metal-chelating agents confer photoperiodic sensitivity on strains of two different *Lemna* species, converting one (*L. perpusilla* 6746) from a daylength-indifferent to a short day plant and the other (*L. gibba* G3) from a plant unable to flower to a long day plant. These results suggest an important role of metal metabolism in the photoperiodic control of flowering, on which further investigations with these and other strains and species are in progress.

Supplementary note as of March, 1961.

The possibility that the chelating agents act by reducing the activity of some metal in the medium, though undemonstrated by reversal experiments, would be supported if lowered concentrations of a metal imitated the chelation effects. Preliminary experiments of this kind failed, but it was clear that medium purification methods as used in micronutrient investigations would be required for definitive results. Such work was deferred in favor of explorations of the "dilution" reversal of EDTA action, which, indeed, seemed to point to an opposite interpretation. However, when Mr. Eric Bachelard (to whom the writer is greatly indebted) observed that the distilled water regularly used in this laboratory was toxic to certain root cultures, it obviously became desirable at least to purify the water immediately. This led to the following results.

In the Hoagland-type medium made with water redistilled from glass, *L. gibba* G3 flowers rapidly in long days and *L. perpusilla* 6746 shows much greater flowering in short than in long days. That is, both plants are now photoperiodic *without* high levels of chelating agents. The conferring of photoperiodic activity by chelating agents thus appears to represent the reversal of an inhibition of photoperiodism caused by a metal ion in the regular distilled water. A number of metals have been tested in the relatively purified system for such inhibition, as assayed by the promotion of flowering in *L. perpusilla* in long days. Both cupric and mercuric ion are highly active; at 1.6×10^{-6} M, the former has no effect on flowering in short

days but promotes the low long-day value up to that found in short days. These results may thus prove significant in analyzing the photoperiodic mechanism by making possible its relatively specific inhibition by known substances. Much further work will be needed to tell how these trace metal effects may be related to the dilution reversal of EDTA action, and whether they are exerted directly or through interference with the action of other metals (e. g., iron). The contaminant in the regular distilled water has not been identified as yet, but is most likely copper; it is interesting to note that the same or an equivalent substance was apparently present in Kandeler's original experiments.

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Photoperiodic control of flowering

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ABSTRACT

The photoreaction that regulates flowering of photoperiodically responsive plants is photoreversible. The wavelength maxima for the opposing reactions are 660 and 730 mμ. The reaction may be written $P(660) \underset{730}{\overset{660}{\rightleftarrows}} P(730)$ in which P(660) and P(730) are the two forms of the photoreceptive pigment. The reaction also proceeds in the direction $P(730) \rightarrow P(660)$ in darkness at a rate in some plants that indicates a half life of approximately 2 hours. It is saturated at low energies in each direction and because of its photoreversible nature it controls flowering equally well at low and high light intensities.

The photoreversible reaction that controls flowering also regulates several other non-periodic photoresponses of plants. These include seed germination, pigment formation, stem elongation, and leaf enlargement, all of which are photoreversed by radiation of wavelengths of 660 and 730 mμ. Because of the identical nature of the photoreactions controlling all these responses, any detail of the reaction may be investigated in the response that best exhibits it. Detection of the reaction controlling stem elongation in albino barley plants, for example, shows that these colorless plants contain the pigment. Because of the conspicuous response of plants to red light, the light-absorbing pigment must be blue. Lack of apparent blue color in albino barley plants, however, indicates that concentration of the pigment is below the limits of color vision.

No chemical assay is known for the photoreceptor, but a physical one based on characteristics of the pigment determined physiologically was designed. It depends on changes in optical density of a sample of plant material or a plant extract induced by moderately intense radiation at 660 or 730 mμ, which converts the pigment to one form or the other. The optical density is then measured at 660 and 730 mμ by a spectrophotometer of special design and sensitivity that uses measuring light beams of such low intensity that they do not drive the reaction perceptibly in either direction. Circuitry is such that the measurement reported is a rapid succession of differences in optical density at the two measured wavelengths. The photoreceptor is next converted to the other form by an appropriate irradiation after which the differences in optical densities at 660 and 730 mμ are again measured. The average difference in optical density measured

with the pigment predominately in one form plus the similar difference when it is in the other form gives a sensitive physical measure of the photoreversibility of the sample.

The method was used to detect the pigment in more than 30 plant species and to follow its separation during extraction procedures. The pigment is a protein, and one form presumably is an enzyme that mediates a presently unknown reaction common to all the phenomena that exhibit photoreversibility. The extracted compound has not yet been completely purified or identified. It was given the trivial name "phytochrome."

The details of this subject were published in more extensive form in several recent papers included in the following list of references.

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Plant Animals as Experimental Tools for Growth Studies[1]

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Some protists, like the hardy, fast-swimming, deep olive-green, photosynthetic, voracious chrysomonad flagellate *Ochromonas danica*, combine the decisive attributes of plant and animal. Most textbook discussions of plant *vs.* animal are fogged by timidity when protists rather than oaks and elephants are discussed. Even the most zoophobic and misanthropic of botanists—only grudgingly granting zoology a status above that of a branch of plant pathology—must concede that in comprehending what *plant* means, insight about animality helps. No less must the student of animality appreciate the plant origins of many of the devices uniquely animal, e.g. vision.

Some morphological and hormonal implications of T. H. Huxley's definition of animals as *eaters* have been sketched elsewhere (Hutner 1961). Electron-microscope and isotope-tracer experiments emphasize that the talent underlying eating is appreciable synthesis of food-vacuole membrane. Organisms unable to do this are plants; they have other specializations: photosynthesis, the elaboration of external digestive enzymes as in heterotrophic bacteria and fungi, or efficient systems for using dissolved nutrients. Since *O. danica* can live by photosynthesis, with all its carbon coming from CO_2 except for those traces represented by its requirements for thiamine and biotin (Aaronson and Baker 1959, Allen, Goodwin and Phagpolngarm 1960), it can live as a plant. It also can live in the dark on particles—i.e., as an animal. Rather than set up a kingdom Protista (Whittaker 1959), and saying "animal-like" or "plant-like," it seems better to call *O. danica* and similar protists *plant-animals*—which accords with their experimental uses. The investigator chooses whether the problem attacked is of zoological, botanical, protistological or protozoological interest; the organism does not care.

[1] As evident from the references, I have acted here as spokesman for a group of colleagues and students but they are not to be held responsible for imprudences in leaping at conclusions. I am indebted to Elliot Packer and Marvin Mendelow for some of the cited data on metabolic loading of *Ochromonas danica*. Work on plant-animals was aided by grants from the Loomis Institute, the American Cancer Society, and grants H-2974 and CY-4182 (C2) from the National Institutes of Health, U. S. Public Health Service.

In trying to decide whether these brown-pigmented newcomers on the biochemical stage are "humanoid," an unforeseen difficulty arises: just what biochemically sets metazoa apart from the many lines of protistan animals? That is to say, the comparative biochemistry of animals is in its infancy. One needs too the background of a comparative biochemistry of basic plant types, comprising photosynthetic bacteria, blue-green, red, and brown algae, and green plants (the latter in the sense of comprising Volvocales, euglenoids, other green algae, and land plants).

This paper outlines some ways in which these plant-animals are uniquely suited for investigating otherwise hardly accessible processes in plant and animal growth. We may so see deeper into *plant* and *animal*.

EXPERIMENTAL ADVANTAGES OF GLUTTONOUS PLANT-ANIMALS. *Ochromonas danica* has an enormous capacity to digest prey. We have watched a few individuals in a hanging drop gorge on dense populations of *Escherichia coli*, nearly wiping out the bacteria while multiplying prodigiously. That ingested bacteria can be nutritionally effective was shown earier in a subtler way: *O. danica* satisfied its biotin requirement when fed bodies of *Thiobacillus thioparus* whose biotin was presumably all bound since it was unavailable to a biotin-requiring coccus (Aaronson and Baker 1959).

O. danica, a glutton, must, like humans in the hunting stage of social development, gorge when it can to prepare for lean times ahead. In gorging, it must cope with surges of fuel and building blocks. We have lately met a problem: What happens when *O. danica* is given *all* the simple building blocks and fuel it can handle—i.e., allowed a sustained gorge? This question is not simple; exploded hypotheses clutter our notebooks. Unexpectedly, at high concentrations of superior substates, e.g., the combination of glycerol and propylene glycol in table 1, growth increases sharply but nutritional imbalances appear. At lower concentrations of substrates (roughly half those in table 1), no such imbalances hinder growth. In unpublished experiments, the additional growth requirements induced in *O. danica* by growth at elevated temperatures overlapped with those induced by media containing methionine far in excess of the concentration satisfying an amino-acid requirement as such. We now think that the common denominator in these "metabolic stress" experiments is a speeding-up of growth which exaggerates the demands for certain nutrients such as trace elements and certain vitamins, and induces lethal imbalances in amino-acid utilization. And, under still greater stress, imbalances develop in the utilization of as yet unidentified lipids.

The *O. danica* results are now outrunning the literature on rat and chick nutrition which guided our early experiments. The appearance of amino-acid requirements under the stress of greatly speeded growth seemingly has its counterpart in the chick, whose high body-temperature (40–

TABLE 1. *Nutritional imbalances shown by* **Ochromonas danica** *in high-substrate media.*

Growth period 5 days. Concentrations of ingredients g./100 ml. final medium unless otherwise indicated.

Basal medium.[a]

KH_2PO_4	0.01 g.	Thiamine HCl	0.05 mg.
$MgCO_3$	0.07 g.	Biotin	0.5 μg.
$MgSO_4 \cdot 7H_2O$	0.02 g.	Fe	1.2 mg.
$CaCO_3$	8.0 mg.	Mn	1.1 mg.
L-Arginine HCl	0.1 g.	Zn	0.6 mg.
L-Asparagine $\cdot H_2O$	0.1 g.	Mo	0.06 mg.
L-Glutamic acid	0.4 g.	Cu	0.06 mg.
L-Histidine HCl $\cdot H_2O$	0.1 g.	Co	0.06 mg.
Sucrose	1.0 g.	B	13.0 μg.
Citric acid $\cdot H_2O$	0.1 g.	V	6.0 μg.
K_3 citrate $\cdot H_2O$	0.3 g.	I	5.0 μg.
$(NH_4)_2H$ citrate	0.1 g.	Se	2.6 μg.

	No. add.	DL-tryptophan 10 mg. + Hycase 0.4	C.S. No. 11 5.0 ml./100 Proteose peptone 0.1 Liver L 0.04 yeast autolystate 0.06
1) Medium 0.6×	2.3	1.6	3.8
2) " 0.8×	2.4	4.6	6.6
3) " 1.2×	3.6	9.4	12.8
4) " 1.6×	0.54	12.4	14.4
5) " 2.0×	0	6.6	7.5
6) NaCl 0.2	0.26	2.2	10.0
7) " 0.5	0	3.0	9.5
8) " 1.0	0	0	0

[a] (Footnotes to Table 1, basal medium)

This portion of the medium was stored frozen as a dry mix. Concentrations of metals are given, not that of the salts. The following supplementary substrates were then added together: Glycerol 0.4 g. + Na_2 glycerophosphate $\cdot 5H_2O$ 0.4 g.

The pH was adjusted to 5.0–5.4. Experimental media were distributed in 5-ml. amounts in 25-ml. Kimble micro-Fernbach flasks, covered with polypropylene caps, and incubated in the dark at 32° for 2 weeks. The basal medium was made up in 2×- or 4×-strength to accommodate supplementation without altering concentrations in the final medium. In the table concentrations are grams/100 ml. unless otherwise indicated. The growth results are expressed in optical density units corrected for dilution effects.

Notes on Table 1. Hycase SF is a low-salt acid-hydrolyzed casein sold by the Sheffield Chemical Company, Norwich, N. Y. Liver L is an enzymatic digest of the 70% ethanol-insoluble fraction of liver (Nutritional Biochemicals Corp.) The "C.S. No. 11" ("complete supplement") is a mixture of Hycase, alkali-hydrolyzed yeast ribonucleic acid, acid-hydrolyzed herring deoxyribonucleic acid, B vitamins, fortified with methionine, tryptophan, adenine, and guanine; for a description see Hutner *et al.*, 1957.

The "accordion" design used in this experiment, in which total concentration of the medium is varied while supplements stay at constant concentration, has proved effective in detecting imbalances. The superiority of more concentrated media, and the elasticity of the osmotic limit in the presence of adequate substrate and amino-acid supplements, may mean that the organism's osmotic pump has one of the highest priorities, and its efficient operation in turn permits still further supplementation of the medium.

42° contrasted with our 37°) and rapid growth makes it outstrip its capacity to synthesize such "unessential" amino acids as glycine and arginine. Also, the appearance of a B_{12} requirement when *O. danica* was given excess methionine + glycine along with certain supplementary nutrients (table 2)

TABLE 2. *Induction of a vitamin B_{12} requirement in* **Ochromonas danica** *by loading with methionine + glycine.*

Basal medium as in Table 1 except glycerol 0.3 g., propylene glycol 0.2 g., + pentane-1,5-diol 0.1 g.

	No add.	B_{12} 5.0 µg.	glycine 0.1 L-isoleucine 0.01 L-leucine 0.02 L-lysine 0.04	C + B_{12} 5.0 µg.
1) No add	2.2	0.95	2.3	1.5
2) DL-methionine 0.1	2.3	1.9	1.8	1.4
3) " 0.2	4.7	2.6	1.4	1.3
4) " 0.4	0	0	1.7	0.86
5) " 0.6	0	0	0	0.13
6) " 0.8	0	0	0	0
7) DL-Methionine 0.5 + glycine 0.1	0	0	0	0
8) " 0.2	0	0	0	0.75
9) " 0.4	0	3.1	1.4	0.95

Notes on Table 2. In many experiments, loading with glycine alone did not elicit a B_{12} effect. The increasing growth with increasing methionine in the unsupplemented medium (column "A"), reflects the use of methionine as a substrate prevailing over interferences with amino-acid uptake or utilization. Use of more complex supplements permits rapid growth with as much as 0.14% methionine. Use of higher levels of substrates, as in the experiment shown in Table 1, changes these balances drastically, as discussed in the text.

may, in view of the multiple interconnections between B_{12} and folic acid synthesis and function (Hutner, Nathan, and Baker 1959), have its counterpart in the enhanced folic requirement of chicks fed excess glycine (Naber *et al.* 1956). The enhanced B_{12} requirements in animals given thyro-active materials and in *Ochromonas malhamensis* grown at elevated temperatures (discussed by Hutner *et al.* 1958b, in a review of the sociology and epistemology of trace-element and B_{12} research) now fit into place as consequences of speeded growth.

Vertebrates, including humans, treated with thyro-active substances, have enhanced nutritional requirements besides that for B_{12}. These nutrients have not yet been identified for the rat (Overby and Fredrickson 1960); likewise, supportive diets for thyrotoxic patients are primitive.

Extensive damage in higher animals is followed by a wasting of skeletal muscle and negative nitrogen balance not alleviated by ordinary high-calorie or high-protein diets. This process is controlled by the anti-shock cortical hormones. If in emergencies higher animals do fall back upon an an ordinarily inconspicuous metabolic pathway to make ready fuel and repair parts, centering around certain amino acids and related compounds,

comparison of metabolic stress in higher animals and protozoa may reveal a common denominator. It becomes of evolutionary and taxonomic interest to look for corresponding systems imbedded in different plant lines. This emergency system may include one or another of the newly discovered metabolic cycles in which vitamin B_{12} figures, such as transformations of propionate and the glutamate → succinate interconversion proceeding by way of β-methylaspartate (Stern and Friedman 1960, Eggerer *et al.* 1960).

In unpublished experiments the added growth requirements induced in *O. danica* by incubation at elevated temperatures resembled those induced by media containing excess methionine. The common feature may be that fast growth increases the demands on certain nutrients (notably certain trace elements and vitamins) and causes imbalances in amino acids.

Unpublished experiments with fast-growing ciliates indicate that efficient substrates can be overlooked because, while giving growth an immediate impetus, essential syntheses lag, especially of amino acids and of vitamins and other co-factors for metabolizing these substrates directly or for preliminary conversions into metabolizable forms. Paradoxically, then, rapid growth may lead to poor growth. Metabolic stresses can be alleviated—and so identified—in organisms amply permeable to nutrients relieving these strains. Otherwise one sees irremediable lethality. Complicated amino-acid balances are no new thing in plant tissue culture. One wonders how many coconut-milk factors are no more then brittle balances. All "protein" and "peptide" factors have so far in microbiology been resolved into amino acids balances, sometimes complicated by peculiar trace-metal requirements or trace requirements for fatty acids or sterols. A particularly instructive example is the ciliate *Glaucoma scintillans* (Holz, Wagner, and Erwin, 1961).

VITAMIN B_{12} AND THE PROTISTAN TAXONOMIC LABYRINTH. The prescient chairman of this meeting has said: "The unifying force of systematics in the fields of botany and zoology gives it a transcendental importance as yet too little recognized" (Steere 1959). The following is adduced in support:

A characteristic separating green plants and higher fungi from other creatures is their lack of B_{12}. Strict vegetarians ("vegans") come down with a facsimile of pernicious anemia unless they are lucky in having intestinal bacteria which are good B_{12} producers. Before regarding B_{12} as the thread of Ariadne that leads through the taxonomic labyrinth of the many protistan groups of puzzling position (how delightful to assign *this* group to the orthodox plant kingdom and *that* one to the main animal lines, just as in Gilbert and Sullivan's *Iolanthe* each child born alive is either a little liberal or else a little conservative), one must understand the distinction between *containing* and *requiring* B_{12}. For surety, an organism neither containing nor requiring B_{12} should be shown to lack any B_{12}-

catalyzed reaction. This caution is suggested by *Euglena:* it requires B_{12} but does not contain any, as measured by all available assay organisms such as higher animals, *Euglena* itself, various bacteria, and *Ochromonas malhamensis*. The taxonomic character represented by B_{12} distribution has economic as well as medical importance: plant feeds for farm animals are now supplemented by "animal protein factor," whose main potent ingredient is B_{12}, usually made by special microbial fermentations. The extremely rapid growth and efficient food conversions permitted by these supplements has helped set the stage for investigations on the importance of nutritional balances for efficient growth; my fellow speaker at this Symposium, Dr. T. H. Jukes, was, with his associates, the pioneer in this field.

Ochromonas malhamensis (smaller than *O. danica* and poorly photosynthetic) has a B_{12} requirement exactly like man's and not like *Euglena*'s; the two patterns are quite different (Ford and Hutner 1955). Our faith that the vitaminic resemblance between *Homo* and *Ochromonas* is more than convergence is encouraged by the remarkable parallel recently becoming apparent to us that all the compounds oxidized by higher animals (as listed by Wilhams, 1959) appear to be utilized by *O. danica*, either when tried singly or when sparked by other substrates. Since, as noted, B_{12} figures conspicuously in stress reactions, any phylogenetic scheme for the different animal lines which lays emphasis on B_{12} patterns needs buttressing by a concordant taxonomy of stress reactions. And so a new taxonomic problem arises: which inducers of stress are the fundamental ones—elevated temperatures, abnormal nutrition, or certain chemotherapeutic agents and herbicides?

ENDOCRINOLOGICAL SEPARATIONS OF PLANT AND ANIMAL. Wooiley (1957) has suggested that serotonin may be the counterpart of indoleacetic acid: that in plants where the sap is acidic, the acidic auxin has evolved; and in animals where the juices are rather alkaline, the corresponding base serotonin operates and, in some lower vertebrates, tryptamine. All, he thinks, may act by altering permeability to Ca and Mg.

Here clearly posed is another problem in finding a common denominator between plant and animal.

The hormones of cellular organisms regulate the choices between using materials as building blocks or as fuel—insulin and steroid hormones being striking examples. If in land plants, as elsewhere, *inter*cellular hormones had their origin in *intra*cellular regulators, then a unicellular green alga, if subjected to metabolic stress, might have its capacity to synthesize hormones so heavily drawn upon that its growth might become dependent on exogenous hormones or hormone precursors. Which green plant to try? Such unicellular form as *Chlamydomonas* or its *Polytoma* have a very re-

stricted permeability. The photosynthetic pigments of the euglenids are nearly identical with those of green algae (Goodwin 1960). In other molecular respects *Euglena* resembles higher fungi, thus it contains ergosterol (Stern, Schiff, and Klein 1960). *Euglena gracilis* is permeable to a fair range of compounds, but it does not ingest particles and so is a poor experimental object as compared with *Ochromonas* or cellular animals for studying nutrition under stress. But the euglenoids include many animals of unmistakable euglenoid antecedents. One of them, *Peranema trichophorum*, in pure culture has nutritional requirements which include any of a variety of "plant" sterols (e.g. sitosterol, stigmasterol, ergosterol) or "animal" sterols (e.g. cholesterol), unsaturated fatty acids, and a wide assortment of water-soluble factors (Storm and Hutner 1953). Its voracity is satisfactory: it can ingest whole small euglenas or cut holes and suck the juices from larger euglenas (Chen 1950). We had found nutritional analysis difficult because it needs surfaces and had narrow tolerances for essential fatty factors supplied according to the knowledge of that time. Recent advances in (*a*) ways of supplying lipid materials; (*b*) knowledge of amino acid balances in nutrition; (*c*) knowledge of the effectiveness of methyl cellulose and like hydrophilic particles which provide surface and so allow 3-dimensional growth in culture vessels; and (*d*) advances in biochemistry, especially in knowledge of switching points between fuels and biosynthetic intermediates—all urge a thorough study of this animal, especially the pattern of its B_{12} requirement.

"CONTROLS" IN ELUCIDATING GROWTH REGULATORS IN PLANT ANIMALS. The remorseless logic of the preceeding argument leads to the conclusion that the proper studies of botanists seeking to identify the intracellular mode of action of plant hormones are plant-animals like *Ochromonas* and animals like *Peranema*—the more voracious and gluttonous the better. Certainly the impotence of the orthodox botanical approaches to the problem, however valuable once in discovering these hormones, means that other approaches are needed; why not an attempt to outflank from the animal side?

In assigning "plant" and "animal" characters to protists, a precept enunciated by an eminent molecular botanist, D. M. Bonner, might be recalled: a biological experiment cannot have too many controls. For the present purposes this means comparisons with as many kinds of organisms as possible before we dare conclude that the common denominator between chrysomonads and metazoa is *the* animal metabolic pattern, and that between the euglenoids and other green-pigmented groups is *the* plant metabolic pattern. Only a few controls can be mentioned here. One wishes that some of the vast and repetitious effort put into studying the metaphytan hormones had been diverted into analyzing growth regulation in the multicellular brown and red seaweeds, and the many kinds of green algae having

multicellular organization. Recent advances in the cultivation of marine algae (Provasoli, McLaughlin and Droop 1957) point at a wide-open field.

Knowledge of the nutrition of other protozoa, notably of ciliates and trypanosomid flagellates, has advanced to where their metabolism under stress is accessible to analysis. Eventually these, it is expected, will be joined by members of the Acrasiales and other groups having ameboid stages in the life cycle. These might be termed plant-animals but in a different sense, since here the emphasis is on the apposition of ameboid feeding stages with immobile reproductive stages culminating in cyst formation. Where these organisms belong in a "natural" classification, whether closer to green-plants and the main animal lines, is wholly unknown and discussion would take us far from the main interests of most of the botanists here.

The dinoflagellate plant-animal line is beginning to be explored biochemically. The possiblities are exciting as they include some of the most elaborate of pure animals, e.g. *Noctiluca*, as well as hosts of photosynthetic forms, with and without phagotrophic abilities.

In a functional sense the symbiotic associations of invertebrates and algal flagellates (classically termed *zooxanthellae and zoochlorellae*) are plant-animals although here the animal hosts play the conspicuous taxonomic role in the association. Again one sees exceptionally succesful forms of plant life almost wholly neglected by botanists. Recent axenic cultivation of some of the zooxanthellae of coelenterates (McLaughlin and Zahl 1959) indicates that this vast assemblage is now accessible to experiment.

PRACTICAL USES OF PLANT-ANIMALS. Determination of B_{12} by *Ochromonas malhamensis* is widely used as a realistic replacement for chick and rat assays; it is the official method in Britain. A new use is developing: screening for anti-cancer agents. Several pharmaceutical firms are employing chrysomonads with some success; details should be published in a year or two; see Hutner *et al.* 1958b.

The patterns of susceptibility to chemotherapeutic agents are in themselves taxonomic characters. The manifold distinctions between antibacterial and antiprotozoal drugs throw into relief the many overlappings between antifungal and antiprotozoal agents. Should *Ochromonas* efficiently detect compounds specifically, cytotoxic to metazoan cells as compared with green-plant cells or, conversely, should *Peranema* detect compounds specifically herbicidal but harmless to chrysomonads and metazoa, the taxonomic scheme inferred from the present discussion would be supported. If plant-animals efficiently detected toxicity to metazoan cells, then they might become useful in detecting those herbicides, insecticides, and other agricultural chemicals presenting unreasonable hazards to higher animals.

Chlorophyll formation in *Ochromonas danica*, *O. malhamensis*, and *Euglena gracilis* is inhibited by the herbicide 3-amino-1,2,4-triazole; as

measured by growth inhibition, *O. danica* and the duckweed *Spirodela oligorhiza* were about equally sensitive (Aaronson and Scher 1960). Aminotriazole inhibition of growth and chlorophyll formation in *O. danica* was annulled by Fe, in part by cholesterol (Aaronson and Bensky 1960), and also by adenine but not by riboflavin; the combination of Fe and adenine was very effective (Aaronson and Bensky personal communication). This instance is cited to indicate how convenient *O. danica* is for mode-of-action studies of cytotoxic materials.

Another source of information drawn on for plant-animal studies is the trouble ruminants occasionally have in coping with surges of ketonic acids. Little if any carbohydrate is absorbed by the cow from the rumen contents; ruminants depend on aliphatic fatty acids. Ruminants may have retained—or developed—metabolic pathways not obscured by the usual predominance of carbohydrate utilization. One of the original reasons for trying propylene glycol on *O. danica* was that it corrected ketosis in cattle as well as glucose did (Waldo and Schultz 1960). The role of corticoid steroid hormones in accelerating interconversions of carbohydrate and other foodstuffs is dramatically shown by their effectiveness in treating ketosis (Neff, Connor, and Bryan 1960).

Screening programs are tedious, expensive but inescapable, and so the most information possible should be derived from them. Detection of cytotoxic materials showing differential toxicity as distinguished from general "protoplasmic" poisons points up a situation which has changed from joke to occasional actuality: a cytotoxic material may be a cure; the trick is to find the disease. The range of test systems might embrace viral, bacterial, protozoal, and fungal pathogens; herbicides; and tumors. Dr. Jukes in this symposium has given examples of how the compounds and concepts of one branch of chemotherapy apply to another. Plant-animals may be versatile tools for identifiying the "disease."

A biochemist told me that plant biochemistry follows two years behind animal biochemistry. Granted that some of this lag reflects the huge support for medical research, the contention here is that plant-animals provide opportunities for botanists to lead; indeed, the first phagotrophic chrysomonads were isolated almost simultaneously by E. G. Pringsheim and R. A. Lewin, both botanists, and my colleague L. Provasoli, whose interests range from insects to seaweeds. *Peranema* was isolated by Chen in Pringsheim's laboratory.

SOME CONCLUSIONS. I should like to endorse explicitly from my own experience, if I may, the importance accorded taxonomy in the previously cited address by Dr. Steere. One moral derived from the previous discussion is that growth studies contribute to taxonomy, and that taxonomy guides growth studies. My colleagues and I had long scoffed at such artificialities as a widely accepted older taxonomic scheme, pro-

pounded by a zoologist haughtily ignorant—or worse, contemptuous—of the work of Pascher, Pringsheim, and other botanists; he welcomed the colorless euglenoids into the animal kingdom and dismissed the green ones as plants fit only for botanists to study. The preposterousness of his judgment was emphasized when streptomycin, in converting green euglenas into permanently colorless ones (put in the genus *Astasia*), proved a lever for moving some euglenas from one kingdom (!) to the other—a feat besides which moving organisms from one species or genus to another paled, so to speak, into insignificance. And, impressed by the unity of biochemistry (before setting out to define disunities of biochemical and chemotherapeutic interest), and partly zoologists by training, we were not too proud to set *Euglena* and later *Ochromonas* to work on medical problems. I therefore enjoy this opportunity to urge botanists not to be constrained by taxonomic divisions reflecting prejudices inherited from bad textbooks and fossilized administrative convenience.

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Recent progress and the goals of plant tissue culture

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Probably the most critical question we may ask concerning plant tissue culture is: What can be done with plant tissue cultures that cannot be done with intact plants? Or, put another way, what advantages are there in growing the cells and tissues of higher plants *in vitro?* The best way to answer these questions is to describe some of the recent work which has been done. In this way, we can evaluate some of the contributions which plant tissue culture has made to increasing our understanding and control of plant functions. We may also be able to discern some of the new avenues of research in plant tissue culture and the goals which lie ahead.

No attempt will be made here to give a complete review of the very extensive literature on plant tissue culture. Gautheret's (1959) recent book summarizes the work up to about 1958 and emphasizes the early pioneering work of White, Nobecourt, Morel, LaRue, and others, as well as his own contributions. Additional important work on root culture (Torrey 1956, Street 1957, Dawson 1960), embryo culture (Rappaport 1954), leaf culture (Steeves and Sussex 1957), ovaries (Nitsch 1951) and other aspects of organ culture will not be considered here. Nutrition of plant tissue cultures and investigations on plant tumors have been reviewed, respectively, by Gautheret (1955) and Braun and Stonier (1958).

TISSUE AND CELL CULTURE. Practically all parts of a plant have been used for tissue cultures: roots, cambium, phloem, endosperm, leaves, pollen, nucellus, and stems. However, it would be misleading to assume that all plants or all tissues of a given plant may be easily cultured; this is not true. Certain groups of plants, the aroids for example, or certain parts of a plant (leaves) are difficult to culture. Since we do know that cultures may be started from a great many plant tissues, eventually it should be possible to induce a tissue culture from any tissue of an intact plant. Tissues from virus tumors, crown galls, insect galls, and genetic and X ray-induced tumors, may also be grown on suitable media.

One of the most important advances in recent years has been the development and use of techniques for growing single cells of higher plants. Muir, Hildebrandt and Riker (1958) took single cells of tobacco callus cultures and established clones of tissue. The cells were grown in the fluid of a nursing tissue culture, but separated from direct contact with the tissue by a

piece of filter paper. This method was put to use by Braun (1959) in an elegant demonstration of the unequivocal totipoteney of single cells of teratomatous crown gall tumors of tobacco. Braun isolated single cells of the tumor, established a clone of tissue, then grafted it back into tobacco plants and obtained normal stems. This showed that the transformation from normal to tumor tissue by the crown gall bacterium was not permanent; hence, the change was not nuclear, but probably cytoplasmic. Rejuvenation of mature single cells of tobacco tissue cultures is reported by Jones, Hildebrandt, Riker and Wu (1960). Using a new technique of microculture they observed that old giant cells were rejuvenated by producing 20–30 small cells within the old cell; the young cells were liberated as a new colony of tissue. Mitochondria and nuclei were also observed during various stages of cell growth and division. Bergmann (1960) made observations on single cells of stem callus cultures of tobacco by plating out the cells on a thin layer of agar in a Petri dish. His method was especially useful for the observation and selection of large numbers of cells. The growth and division of single cells of tobacco tissue was readily followed in culture. Use of this technique should assist in the establishment of clones of tissue derived from single cells. Suspensions of carrot root cells growing in liquid media were studied by Steward (1958), Steward, Mapes, and Mears (1958), and Mitra, Mapes, and Steward (1960). They described the development of whole carrot plants from these cell cultures. The plants grown *in vitro* were transferred to the greenhouse and grown to flowering and seed production. The remarkable observation in their work was that the development of carrot plants from cultured cells resembled the typical pattern of embryogenesis in carrot.

To all outward appearances most cultures of plant tissue appear to be homogeneous masses of undifferentiated cells. Closer observation reveals that some cultures contain only random vascular elements in the callus and others produce normal or abnormal roots and occasionally abortive stems or buds from callus tissue. Also, at the cellular level, the chromosome complement has been found to vary, depending on the culture medium. Torrey (1959) found that tissue cultures from pea root callus, grown on media containing 2,4-dichlorophenoxyacetic acid and yeast extract, changed from diploid to tetraploid within one week; cultures grown on a synthetic salt medium without additives maintained their diploid complement. This tendency to polyploidy has also been described in spruce tumor tissue cultures by de Torok and White (1960) and for other tissues as well.

Grafting tissue cultures into whole plants, or grafting buds into tissue cultures, may prove helpful for investigating how plants control tissue function. Successful grafts of endive buds into endive root tissue cultures have been made by Camus (1949); the vascular elements of the buds and the tissue culture were united. However, Wetmore (1954) found that this

union of vascular elements was absent in grafts of lilac buds into lilac callus. The effect of reciprocal grafts of tissue cultures from two different species was reported by Duhamet (1957). With radish tissue culture as the stock, wild salsify tissue (scion) was stimulated in its growth; conversely, with wild salsify as the stock, radish was inhibited in growth. Results of this type suggest that certain biochemical and genetic aspects of graft incompatibility might profitably be studied *in vitro*. This is a corollary to the over-all problem of how differentiation of structure and function may be brought about in tissue cultures under the influence of specific chemicals or substances from the whole plant.

One of our own current interests is the culture of tissues from the pollens of higher plants. Such tissues have been obtained from the pollen of the gymnosperms *Ginkgo biloba* (Tulecke, 1957), *Taxus* sp. (Tulecke, 1959) and *Pseudolarix amabilis*. Thus far, no angiosperm pollens have been induced to proliferate as tissue cultures. The pollen tissues are haploid, undifferentiated, and may be colorless or contain chlorophyll. One strain of *Ginkgo* pollen tissue was isolated on a medium which contained the amino acid arginine (Tulecke, 1960); it grows best on this medium, but ammonia, lysine, urea, or proline can substitute for arginine to some extent. The *Taxus* tissue is white and it has given rise to a variant which is green; the *Pseudolarix* tissue is readily derived from pollen and it is green but slow growing. The derivation of tissue cultures from the haploid male sexual apparatus—the pollen grain—makes these tissues of particular interest. They may contribute to our understanding of pollen physiology and products of their metabolism are of interest. The tissues also offer the possibility of establishing homozygous lines of plants not attainable by other means. Diploidization, grafting the tissues into intact plants or chemically inducing regeneration would be necessary. Because such plants would reveal recessive male characters, they would be useful in horticultural and agricultural breeding programs. They would also shorten the time required to attain homozygosity of a particular strain.

Plant tissue may be cultured in large quantities in liquid media in a manner similar to that used for microbes and animal tissue cultures (Tulecke and Nickell 1960). The tissues of *Ginkgo* pollen, *Rosa* stem, *Ilex* stem, and *Lolium* endosperm have been grown in a carboy culture apparatus (10–18 liter capacity) of simple design. Aeration and agitation by a stream of sterile air, together with facilities for medium additions and sampling, meet the necessary requirements. The yields of tissue amount to two to five pounds or more per carboy, depending on the tissue, the age and amount of inoculum, the temperature, the length of time the carboy culture is run, and the level of nutrients supplied.

MEDIA, GROWTH FACTORS, AND GROWTH REGULATION.

A rather large number of media have been devised for the nutrition of plant tissue cultures. Some of these are synthetic (Gautheret 1959, Heller 1953, Nitsch 1951, White 1943, and Hildebrandt, Riker and Duggar 1946) and others contain mineral elements with non-defined adjuncts such as yeast extract, coconut milk, or tomato juice. The most desirable situation from an experimental point of view is to have a medium of known constituents which will support minimal (or better) growth. However, in those cases where tissue growth depends on yeast or some other extract, an analytical approach to medium design is often adopted, the purpose being to isolate the components of the extract which promote growth. The fractionation of coconut milk by Shantz 1954, Pollard, Shantz, and Steward 1959, and Shantz and Steward 1955, has revealed a variety of substances which promote the growth of explants of the phloem of carrot roots. Amino acids, sorbitol, 1,3-diphenylurea, leucoanthocyanin, and several unidentified substances have proved active in their bioassay. Other growth factors in tomato juice are reported by Nitsch (1951a) and Straus (1960), those in corn steep liquor by Fox and Miller (1959). Whether these factors are essential to one or many tissues or to a particular cell function, remains to be determined.

Synergism is known to occur between the different compounds in a medium upon which a tissue culture grows. An example is that reported by Steward and Caplin (1951) for 2,4-D and coconut milk. Potato tuber tissue was stimulated to rapid proliferation on a basal medium containing added 2,4-D at 6 p.p.m. and coconut milk, 18 per cent but very little growth occurred on media containing only 2,4-D or only coconut milk. This stimulatory effect of 2,4-D and coconut milk has been used by investigators to grow many plant tissues *in vitro*. Unfortunately, the nature of the substance(s) in coconut milk which interacts with 2,4-D to stimulate plant growth has not yet been identified.

The discovery of kinetin (6-furfurylaminopurine) and the demonstration of its role in the regulation of root, callus, or shoot production in tobacco pith callus has been reported by Skoog and Miller (1957). On a basal medium with 2.0 p.p.m. IAA, tobacco callus cultures formed roots with 0.02 p.p.m. kinetin, callus with 0.2 p.p.m. kinetin, and shoots with 2.0 p.p.m. kinetin. Different strains of tobacco callus were found to require adenine and/or tyrosine in addition to kinetin and IAA. Skoog and Miller emphasized that the specific responses obtained would not apply to all tissues. Their results also oppose the thesis of specific organ-forming substances.

In the past, much effort has been placed on increasing the growth rate of plant tissue cultures. Better media, new growth-promoting compounds,

the use of liquid media, and so on, have all aimed at improved growth. The results have been good as very appreciable yields of tissue can now be obtained. However, it is quite certain that this approach is undesirable for certain results, for example: differentiation, attaining a metabolic state for the synthesis of certain compounds, or for virus replication (Wu, Hildebrandt, and Riker 1960). In these instances, a reduction in growth rate might be required to achieve a function analogous to that in an intact plant. The use of antimetabolites is one method which has been tried with limited success, although it does assist in identifying metabolic pathways in operation in a tissue. Steward, Pollard, Patchett, and Witkop (1958) used hydroxyproline and several related compounds to inhibit the growth of carrot root tissue cultures; this inhibition was reversed by the addition of proline to the medium. These results provided another piece of evidence for the theory of Steward and Pollard (1958) that proline is taken up in a portion of cellular protein which does not turn over; this proline is transformed into hydroxyproline in the protein *in situ*. Actively dividing cells are thought to contain more of the protein with hydroxyproline than resting cells.

ANALYSES AND BIOCHEMISTRY. Comparisons of plant tissue cultures with the parts from which they were derived have been made on a number of tissues: Bové, Bové, and Raveux on *Citrus limonum* (1957), Steward, Thompson and Pollard on *Solanum tuberosum* (1958), and Weinstein, Nickell, Laurencot, and Tulecke (1959) on *Agave toumeyana*. Striking differences were observed in each case. Tissue cultures of *Citrus* contained more γ-aminobutyric acid, proline, citric acid, and an unidentified organic acid than did the intact plant; the level of potassium in the culture medium strongly influenced the amounts of free amino acids in the tissue cultures. For potato, the protein of actively dividing cells in tissue culture was found richer in hydroxyproline and basic amino acids than the protein of mature potato tuber cells. The bulk protein of *Agave* also showed a greater amount of hydroxyproline in the tissue culture and free hydoxyproline was present in the tissue culture but not in the leaves. Thus, the transformation of *Agave* from a slow-growing leaf tissue to a fast-growing tissue culture involved the conversion of proline to hydroxyproline in protein synthesis.

Cell particulates isolated from plant tissue cultures have begun to attract the interest of biochemists. Actively growing cultures can provide an excellent source of mitochondria (Tamaoki, Hildebrandt, Riker, Burris, and Hagihara 1960), cell walls (Lamport and Northcote 1960), and protein particles (Sutcliffe, Bollard, and Steward 1960). Dougall and Shimbayashi (1960) studied fractions of tobacco pith callus tissue cultures, including soluble amino acids, soluble protein, formic acid soluble protein, and cell wall residue. After several washing cycles, the cell walls of tissue cultures

were found to contain 21 per cent of the total nitrogen as compared to 1.7 per cent for cell walls from pith cells of tobacco stems. In the cell wall residues of the tissue cultures, the predominant amino acid was hydroxyproline; this amino acid was not identified from the other cell fractions. This suggests that hydroxyproline is a structural component of cell wall protein, especially in actively dividing cells. Supporting evidence for this view comes from a report by Lamport and Northcote (1960) who found hydroxyproline in the protein of cell walls from tissue cultures of *Acer pseudoplatanus* and *Phaseolus vulgaris*. In fact, the correlation of hydroxyproline content and amount of cell wall material was sufficiently good for hydroxyproline to be an index of the cell wall material in tissue cultures. On this basis, *Acer pseudoplatanus* cells are made up of 40 per cent cell wall at the stationary phase of growth, hence, the cell wall protein is an important component of these plant cells.

The free amino acids of crown gall tissues and normal tissues of wild salsify have been analyzed qualitatively by Lioret (1957). Surprisingly, an unknown free amino acid was found in the crown gall tissue which was not present in normal tissue. A large amount of this amino acid was also reported for crown gall tissues of tobacco, Jerusalem artichoke, and Virginia creeper.

The amino acid arginine occurs in large amounts in the winter storage tubers of the Jerusalem artichoke. Tuber tissues were cultured *in vitro* by Duranton for the purpose of studying the metabolism of arginine through the use of labeled compounds (1958). The scheme of arginine breakdown in tuber tissue was found to resemble the same degradation pattern known to occur in microorganisms, i.e., from arginine to urea and ornithine and thence through intermediates to proline. Results on the synthesis of lysine by tissue cultures of *Helianthus*, *Melilotus*, *Agave*, and *Ginkgo* and whole plants of *Azolla* and *Lemna* (grown *in vitro*) are reported by Vogel (1959). The pathway of lysine synthesis was via *a*- (-diamino) pimelic acid, the same as that which occurs in *Escherichia coli* and the algae.

MIXED CULTURES. Plant pathogens and their relation to host tissues have been studied *in vitro* through the use of plant tissue cultures. The work on genetic, virus, and crown gall tumors has been reviewed by Braun and Stonier (1958). One of several important results of their work on crown-gall tissues was the demonstration that both a cell division and a cell enlargement factor were produced by fully transformed autonomous bacteria-free crown gall tissues of tobacco. Transformation of normal cells was a function of the time the tissue was in contact with the virulent bacterium: 34 hours gave slow-growing tissues, 50–60 hours gave moderately fast-growing tissues, and 4 days were required for the production of fast-growing tissues. In contrast, the normal tobacco tissue did not grow *in vitro* unless supplied with kinetin and an auxin; this growth was very slow.

Growth equal to that of the fully altered type of tumor tissue was achieved only when the normal tobacco tissue was grown on the basal medium supplemented with glutamine, asparagine, inositol, and guanylic and cytidylic acids, in addition to the auxin and kinetin.

Root knot nematodes were found by Sayre (1958) to grow better in potato callus which contained vascular elements than in undifferentiated callus. Females were always associated with vascular tissue; their development did not go beyond the second larval stage when vascular tissue was absent. Actively-growing tissue cultures were least attractive to these nematodes. The growth of nematodes on tissue cultures offers the possibility of studying how the feeding habits of the nematode cause polynucleate giant cells to form in the host. It also provides a means of establishing the growth requirements of nematodes and, hence, could lead to the development of improved nematocides.

The growth of plant parasitic fungi on tissue cultures of grape has been studied by Morel (1948). Both a powdery mildew, *Uncinula necator* (Schw.) Burr., and a downy mildew, *Plasmopara viticola* (Berk. and Curt.) Berl. and de Toni, were successfully grown *in vitro*.

Some encouraging results for the replication of plant virus have been presented by Bergmann (1959) and by Wu, Hildebrandt, and Riker (1960). Bergmann (1959) obtained yields of tobacco mosaic virus (TMV) from *Nicotiana tabacum* tissue which equaled that found in systemically infected tobacco plants. However, the production of virus was not consistently high. In the results of Wu and associates, TMV was produced in greatest amount from tobacco tissue cultures which were 30–40 days old when inoculated with virus; the virus was titered one week later. The younger actively-growing cells were more resistant to virus infection.

Callus cultures from grape galls have been used to raise gall-forming phylloxera. Pelet, Hildebrandt, Riker and Skoog (1960) note that one colony was kept alive through five successive generations. They found that the gall tissues differed very markedly from normal tissues in their response to 1-naphthaleneacetic acid, kinetin and other substances.

ENZYMES AND SYNTHESES. Studies on cultures of virus wound tumor tissue of *Rumex* have shown that α-amylase is secreted into the culture medium by the tissue (Brakke and Nickell 1951, Nickell and Brakke 1954). This was the first demonstration of an extracellular enzyme from higher plants. Excised roots of *Rumex* showed no detectable enzyme secretion. The lack of a virus-free normal tissue culture of *Rumex* made it impossible to compare the virus induced tumor tissue with a normal tissue. Hence, it is not known whether α-amylase secretion is attributable to the virus in the tissue or to changes induced by the virus. However, other tissue cultures are known to utilize starch as the sole source of carbohydrate; Straus and

LaRue (1954) reported starch utilization by tissue cultures of corn endosperm.

Gentile and Naylor (1955) determined the presence of peroxidase, catalase, and two cytochrome-c reductases for *Rumex* virus tumor tissue; ascorbic acid oxidase, polyphenol oxidase, and laccase were absent. More recent biochemical studies of the *Rumex* tumor tissue are in progress by Porter and Weinstein (1959).

There are relatively few reports on the synthesis by tissue cultures of compounds which they might be expected to make. A tissue isolated from the root of a plant which produces an alkaloid would be expected to have the capacity to synthesize that alkaloid. West and Mika (1957), for example, demonstrated the synthesis of atropine by callus cultures from roots of *Atropa belladonna*. The yields were quite low, however, when compared to the intact plant; they were also lower for root callus as compared to root cultures. The change from differentiated tissue and organs to callus tissue appears to involve the loss of the ability to synthesize certain compounds. Whether growth and a particular metabolic pathway may be regulated to give desirable end-products remains to be seen. Efforts and results in this direction can be expected from investigations on tissue cultures of drug plants.

PROBLEMS OF CULTURING PLANT TISSUES. To keep a proper perspective, some of the difficulties of culturing plant tissues *in vitro* should be mentioned. For example, one to six months is needed to establish a vigorous tissue culture; additional time may be needed to design and improve the culture medium. The necessity of carrying out most manipulations under sterile conditions is sometimes troublesome as in the growth period of two to four weeks for many experiments. However, there is an even greater difficulty which also applies to the field of animal tissue culture (Biggers, Rinaldini, and Webb 1957). This is the tendency of tissue cultures to converge to a common cell type, to narrow their range of enzymes and metabolic activities, and to lose their specific functions. These difficulties, which are sometimes cited as general criticisms of plant tissue culture, often seem to be added to those inherent in a specific problem. The fact that advances have been made in the face of these difficulties is the best indication that the methods and approach of plant tissue culture are sound.

CONCLUSIONS. The methods of plant tissue culture now enable us to grow single cells or large amounts of tissues from various parts of higher plants. These cells and tissues are useful for a wide range of experimental studies; they may be used for cytological, physiological, biochemical or morphogenetic problems. Radioisotopes, antimetabolites, cell particulates, analytical procedures, microdissection, grafting, and other procedures have been used in various combinations to help solve some of the basic problems

of the growth and function of plant tissues. It would be surprising indeed if the advances of the last decade were not greatly surpassed by future endeavors.

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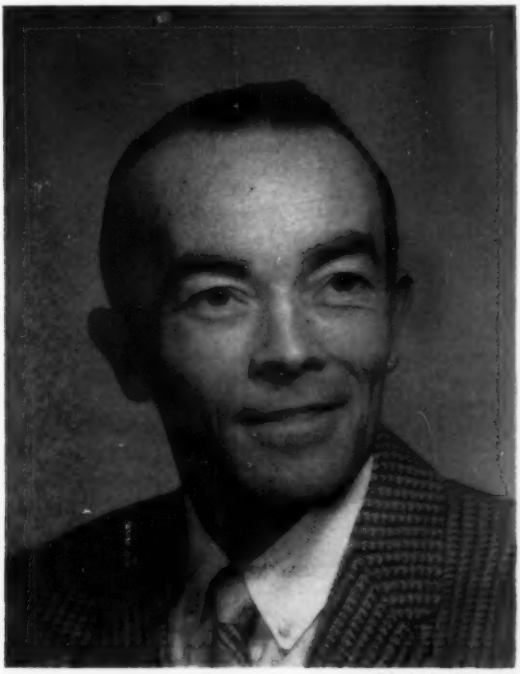
TORREYA

David Fables Jr.

James Leland Edwards

When David Fables died on February 22, 1961, the Torrey Botanical Club lost one of it's most active and enthusiastic students of the local flora. The importance of this severe loss to the organization becomes strikingly apparent when the relatively small number of active field botanists in the local region is considered and also the fact that Fables' activities and interests, although mostly confined to New Jersey, covered a large part of what has been established as the Torrey Botanical Club's local area.

David Fables, who died at the age of 43, was born in Bayonne, New Jersey. He lived in Roselle Park and Roselle, New Jersey for many years. His educational training included Roselle Park High School, Union Junior College at Cranford, then Seton Hall University for a B.S. degree in 1942 and a Master's Degree in Biology in 1947. During his Union Junior College years and later years while working for his Master's Degree, he worked as a laboratory assistant in Union Junior College, as a biology teacher at



(Photo by Bogard)

DAVID FABLES, Jr.
May 24, 1917—February 22, 1961

Seton Hall Preparatory School, and at St. Patrick's High School in Elizabeth. He joined the faculty of Union Junior College in 1946 and eventually became the head of the Biology Department there. He was so popular with the College that a Memorial Fund to be used mainly to develop a natural area on the College property has been established in recognition of his contributions to scientific education. Dave continued graduate studies at Columbia University and later at Rutgers leading toward a Doctor's degree in Biology. Twice he received grants from the National Science Foundation, first for summer work at the University of Oregon working on marine biology. The second covered studies in the New Jersey pine barrens, particularly in the Wharton Tract—now state owned and including much of the heart of the "barrens".

Torrey Botanical Club members who went on field trips had many chances to meet and know Dave because he served on the Field Committee from 1946 to 1955 and was chairman in 1954. He also participated in New Jersey Audubon Society trips and the annual "Branchville" meetings.

He was in the field more than is realized. His teaching duties at Union Junior College and his health, which was not always even reasonably good, interfered a great deal. Certain areas were given more attention than others. The pine barren country near Lakehust, where he could live with relatives, became well known to him. With the help of his students he traversed the Appalachian Trail across New Jersey and listed the plants observed. Some spots particularly interested him such as, the White Cedar Swamp in High Point State Park, swamps near Greendell; Quaker Bridge, and Oswego Lake in the pine barrens; the cliffs along the Delaware River above Milford in Hunterdon County. He published his findings at the last place in Torreya in 1951.

His interests in the field were by no means confined to the flora. All animals were noted. He was one of the original group who formed what is now known as the Urner Orithological Society, and became the first editor of its publications. When the need for an up-to-date list of New Jersey birds became evident, the Urner Club members assembled their personal records and David Fables characteristically volunteered to follow through. The Annotated List of New Jersey Birds was published with David Fables as author. Because of his own interests, perhaps because in teaching biology he had to cover many phases of life or perhaps because he sensed the need of a more comprehensive gathering of people whose interests included many "ologies" he formed the New Jersey Field Naturalists. For this group he edited a publication called "Caesarian Flora and Fauna" and also for this group published his findings on trips along the Appalachian Trail. Recently he had begun to assemble information about the native orchids of New Jersey. Some of his friends hoped that he would eventually tackle the job of writing a "Flora of New Jersey".

David Fables found time to accomplish many things and belonged to many organizations. To some people he will be remembered for his newspaper articles published monthly and called "Afield in New Jersey" or for his contributions to the publications of the New Jersey Audubon Society, or for his Breeding Bird Censuses and Christmas Census in the publications of the National Audubon Society. The people who went with him on any of his types of field trips certainly have the best of this situation because they knew the man personally. His variety of interests eventually led to his becoming a sort of clearing house for people to turn to when they wanted to find out "who was doing what" and what the latest news might be.

In the educational field, his ability to interest his students was very superior. Perhaps at this point a part of a tribute to him by one who knew him at Union Junior College might be quoted. The words are by Saul Orkin. "He taught inspiringly, and his students responded in kind. They learned, as Dave often professed, that science was not sterile and mathematical, but warm and poetical. He listened easily, and his sympathetic ear made him the best of counsel. Many who could go no farther under their academic burdens sought his advice. Whatever he said, the effect was animating, for they often continued, improved, and sometimes excelled."

Field Trip Reports

May 21, 1961. Marine Park, Brooklyn, New York. Only 45 species of plants were observed, owing partly, no doubt, to a cold, wet spring, which seemed to have retarded plant growth somewhat. Only three species had not previously been encountered in Kings County by the writer. A clump of *Sedum purpureum* (Liveforever) was seen growing on a sandy wasteland, a gnarled *Prunus persica* was blossoming by the roadside (possibly the result of a peach stone thrown from a passing automoble), and a last-year's seed stalk of *Verbascum thapsis* was observed on a sandy knoll. Other species seen included *Cerastium semidecandrum*, *C. viscosum*, *Stellaria media*, *Arabidopsis thaliana*, *Sisymbrium altissimum*, *Potentilla norvegica*, *Medicago lupulina*, *Lamium amplexicaule*, *Linaria vulgaris*, and *Veronica arvensis;* these have not previously been reported from Kings County. Two other species encountered, *Geranium pusillum* and *Poa annua*, appear in the New York State Museum check list of the Kings County flora. The remaining 30 species seen on this trip have been listed in previous reports. Attendance, 6. Leader K. L. Brooks.

June 4–7. Appalachian Trail Conference, Delaware Water Gap, Pa. The triennial meetings of the Conference were well attended with delegates from clubs all along the 2000 miles from Georgia to Mt. Katahdin, or vice versa. Maps, guidebooks, camping, hiking, and trail literature were displayed. Some excellent photographs made along the Trail were on display. An attractive panel showing trail maintenance equipment was a not so subtle reminder of the effort that goes into making the Appalachian Trail possible. There were lectures Sunday, the business meeting Monday and hiking every day—bird hiking, botanical hiking, and just plain walking. The Torrey Botannical Club, a member through the New York–New Jersey Trail Conference, is entitled to two delegates—two votes. Individual memberships are also welcomed by both conferences. The 1964 meetings will be around Manchester, Vermont. Start planning.—John A. Small, Delegate to N. Y.–N. J. Trail Conference.

June 18. Appalachian Trail, Warren-Sussex Counties, N. J. Our section of the Trail received a "poor condition" report in Appalachian Trailway News this spring. It was probably deserved. Three or four people, or half a dozen, cannot travel the long distance and get a lot of work done in a day. Repeated pleas have repeatedly gone unheeded by those able to pull a saw, swing a weeder, or use heavy pruning tools. This year we removed all of the big logs and repainted the blazes but one weeder and two pruners were not enough to deal adequately with the grass, brambles, and scrub oak besides properly trimming the overhang.

Over a dozen trips in our schedule this year propose to use trails. These trails do not maintain themselves. Surely the upkeep of a 4½ mile section, 1½ miles of which is on dirt road, is not too much responsibility for those of us who participate in the field program, but who wants to appear as leader in caring for a trail that can only be reported as unsatisfactory? Attendance 6. Leader, John A. Small.

June 25–28. Syracuse vicinity, New York. Our annual participation in the foray of the Northeastern Section of the Botanical Society of America was up to par or better, as usual. This was the 12th such excursion in the 15 year history of the Section. T. B. C. has been well represented each time and reports of most of the forays may be found in appropriate volumes of the Bulletin—Torreya section. A special attraction this year was "Checklist of the Vascular Plants of Onondaga County, New York" recently completed by Dr. Mildred E. Faust and published as Bulletin of the Syracuse University Museum of Natural Sciences, No. 9.

Dr. Norman J. Gillette, Chairman of the Section, greeted us Sunday evening and introduced the local committee, our caretakers for the next three days: Dr. Faust in charge of field trips; Dr. Paul Sedgwick to address us and show excellent color photo-

graphy—time lapse movies and stereoviews—Monday evening; Dr. Lillian Sedgwick a constant source of botanical information—habitat or nomenclature; Boyd R. Greenwalt a competent Volkswagon pilot and mess sergeant—graduate student; and Eva Van Sanford also a graduate student of much assistance with the chores. Unofficially there were wives with immense botanical ability as attested by the refreshments provided.

No attempt will be made to produce a list of plants seen though very adequate and extremely useful lists were in hand as we visited the several areas. Syracuse has been known for its salt flats but we found these much changed—botanically for the worse—since the A.A.A.S. summer meeting in Syracuse in the 1930s. It will be too bad if the remaining fragment cannot be preserved as a portion of the park system around Onondaga lake or as a natural area. The Peter Scott Swamp enabled us to see the complete successional series from open water to swamp forest (elm). We explored Sand Ridge Bog examining another successional series from open water said to be over 29 feet deep to tree covered areas of arbor vitae, larch, red maple, pine, and spruce. Spore producing sphagnum was common. The terrain ranged from kettleholes to drumlins and on one mesophytic hillside we examined the oak-chestnut-tulip tree forest, finding *Magnolia acuminata* as a rarity.

Tuesday, in Clark Reservation, we reviewed a great variety of plants, particularly ferns, and climaxed by the hart's-tongue fern. White Lake with water of remarkable clarity, *Chara*, and marl, afforded a very satisfactory study of swamp forest. In each such locality a most interesting mingling of northern and southern species could be observed. The bog and swamp species of far northward range were particularly interesting.

Wednesday morning we took a quick view of the beech-maple-hemlock-yellow birch forest and then moved to Punkin Hollow, a marly arbor vitae area with a terrific display of showy lady's slipper and other orchids. This was a heyday for the photographers, who had not been idle before. Then bon voyage.

At the business meeting Tuesday evening, Dr. G. Fred Somers invited the Section to the University of Delaware for the 1962 meetings, in mid June. Officers elected were Dr. Somers, Chairman; Dr. Donald Huttleston, vice-chairman; and Dr. William Niering, secretary-treasurer. 1961 attendance was 50+. Reported by John A. Small.

BOOK REVIEWS

Introductory Botany. By Arthur Cronquist. 1–873 pp. Harper & Bros., New York. 1960. $9.25.

For those who teach full-year general botany courses and who prefer the taxonomic approach, Dr. Cronquist's book will be of particular interest. It is handsomely printed and is copiously illustrated with excellent photographs and line drawings. A large proportion of the drawings were taken from other texts, but the author has chosen them with care. The book contains a glossary, which the student should find helpful.

After five introductory chapters (78 pages) the author devotes seventeen chapters (371 pages) to a consideration of the plant kingdom, from the bacteria through the coniferophytes. The next eleven chapters (257 pages) consider the angiosperms, and the last four chapters (105 pages) deal with heredity, evolution, and ecology. An elaborate 30-page key to the "Divisions, Classes, and Orders of Plants" follows these.

The taxonomic approach in college botany texts has been rarely used in the past few decades, and it is encouraging to see several such books published recently. In my opinion it is more educational than a biochemical or angiosperm approach. Dr. Cronquist points out in his Preface that ". . . it permits a natural evolutionary sequence and . . . few entering college students have more than a superficial familiarity with the structure, functions, and reproduction of angiosperms." In addition he notes that a knowledge of the life cycles of simpler plants is necessary to an understanding of the angiosperm life cycle. The same may be said for structure and function.

In arranging the various groups of plants, Cronquist utilizes a system mainly of

his own devising. This I find very questionable procedure. Perhaps Dr. Cronquist's scheme and its evolutionary implications may someday be recognized as being more "correct" than other current schemes. But at present it cannot be so evaluated. Many instructors may not wish to have their students learn a new relatively untested classification, preferring instead a text with a more traditional approach. Throughout the chapters on the plant groups, Cronquist argues his case for his scheme. This seems to me out-of-place in a general text, as it often necessitates the introduction and discussion of quite technical information, as well as a great deal of speculation. Dr. Cronquist's proposals and arguments may be of great interest and pertinence to professional taxonomists, but I do not believe college freshmen will profit from them.

Cellular physiology and genetics have relatively short treatments. One chapter (27 pages) covers heredity; physiological processes are mentioned briefly in scattered references throughout the text. These are very serious omissions in a text intended for the first course in botany.

In summary, Dr. Cronquist and Harpers have produced an attractive, scholarly textbook, which may find considerable use in elementary plant morphology courses and in general botany courses where the instructor is willing to accept Cronquist's taxonomic approach. The instructor will need to add some supplementary material, particularly in cellular physiology.—NORMAN H. RUSSELL.

Flora of the Santa Cruz Mountains of California. A Manual of the Vascular Plants. By John Hunter Thomas. 434 pp. 250 figs. 10 habitat photographs, Stanford University Press, 1961. $8.50.

The Santa Cruz Mountains are a natural area in the outer Coast Ranges of California, including the city of San Francisco and extending southward from it for about 74 miles. From the approximately 1400 square miles of this area are recorded 1662 species of vascular plants, 535 of them introduced; many of the latter are found only in close proximity to cultivation.

Local floras have a special significance in the study of the flora of California, since they present one solution to the problems presented by the large size and complexity of the State's flora. They are not only important as records of the rapidly changing natural scene, but are also indispensable as aids in teaching systematic botany. The numerous line drawings (from Abrams' *Illustrated Flora of the Pacific States*), excellent keys, and glossary of the present volume should enable the user to identify the plants of the area readily, despite the lack of descriptions.

Stanford University Press is very much to be congratulated on the appearance of this sturdy and attractive book. Ten photographs illustrate the helpful discussion of the ten major plant communities of the area, and one of them (taken by W. R. Dudley near the turn of the century) is reproduced as a frontispiece and on the dust jacket. In addition, the author has provided a concise description of the area accompanied by a clearly drawn map and convenient index of place names, with a section on the geology by E. E. Brabb.

This book is almost entirely free from typographical errors. Its conservative nomenclature is, in general, that of the standard State and regional floras. For most species, only the habitat and local range are given, but for a few there are additional pertinent comments. In at least the cases of *Plantago erecta* (p. 322) and *Stephanomeria virgata* (p. 341), the remarks made tend to dismiss on insufficient evidence two groups which are badly in need of biosystematic study. An alert student investigating the plants of the area with the aid of this book would soon discover many other groups as richly deserving of detailed investigation.

The Coast Ranges of central California are a meeting place for northern and southern plants, with those from the north generally exending down the outer, moister ranges, and those from the south ranging northward in the relatively dry inner ranges. About twice as many species (164) reach their southern limits in the Santa Cruz Mountains as in Marin County (89), just across the Golden Gate to the north, which

again serves to emphasize the relatively small historical importance of San Francisco Bay as a barrier to plant migration.

In summary, this handsome volume is a substantial contribution to our understanding of the flora of California. As such, it will prove useful not only to local students but to all interested in the complex floristics of western North America.—PETER H. RAVEN, Rancho Santa Ana Botanic Garden, Claremont, Calif.

Checklist of the Vascular Plants of Onondaga County, New York. By Mildred E. Faust. (6) + 85 pp. Bulletin of the Syracuse University Museum of Natural Science No. 9. Syracuse 10, N. Y. 1961.

Aside from the challenge of making additions, a checklist, if it be reliable, is a very useful tool to the resident botanist of any locality. It is even more useful to the serious botanical visitor. The present checklist must set a standard for reliability. The author is a careful, consistent, long time student of the county's flora. She has the advantage of being an ecologist. She has studied and incorporated the earlier floral publications of the county and adjacent districts. The entries are substantiated by vouchers in the herbarium of Syracuse University, together with many in the herbaria of the College of Forestry, and the New York State Museum. All entries have been checked by another competent botanist, Dr. Stanley J. Smith of the New York State Museum. Plants of doubtful naturalization are indicated as are those for which there has been no record in the past quarter century. The entries total 126 families, 598 genera, 1622 species, and 1662 subspecies and varieties. There is an index to genera but no keys, descriptions, or annotations. Names of the plants in the various taxa are those currently condoned by the New York State Museum. The authority is given and synonomy seems to be adequate. Common names are from various sources, selected in accordance with local usage. There are possible arguments here no doubt. One may also find occasion to reflect upon the subspecies and the variety, which are here given equal rank. But of course one may always reflect upon the Rules of International Botanical Nomenclature.

Congratulations to the University's duplicating office, to the Museum of Natural Science, and to Dr. Faust on a job well done.—JOHN A. SMALL, Douglass College, Rutgers—The State University of New Jersey.

The Meaning of Wilderness to Science. Proceedings, Sixth Biennial Wilderness Conference. Edited by David Brower. Sierra Club, San Francisco, xi + 128 pp. 92 plates. 1960.

The Sierra Club has long been a leader in accenting the need for conservation in America. Founded in 1892, it has given stress to a program of "study and protection of national scenic resources." Although the Club is most closely concerned with conservation problems in the western cordillera, the monthly Sierra Club Bulletin and the many other publications by the Club have been increasingly effective in arousing federal agencies, private organizations and the general public to an awareness of the importance of conservation.

One of the more recent books to be published by the Club, "The Meaning of Wilderness to Science," makes a case in point. This book covers the Proceedings of the Sixth Biennial Wilderness Conference which was sponsored by the Sierra Club and received the cooperation of several other organizations. The Conference, held in San Francisco on 20, 21 March 1959, had as its principal speakers, Daniel B. Beard, Stanley A. Cain, Luna B. Leopold, Robert Rausch, Ian McTaggart Cowan, Raymond B. Cowles, and Frank Fraser Darling. The book carries their addresses, a foreword by the Club's Executive Director, David Brower, the opening welcome of President Harold C. Bradley, and a reprinting of a pertinent lecture by G. M. Trevelyan, entitled "The Call and Claims of Natural Beauty," delivered in London in 1931. Forty-eight pages of the book are given over to photographs, many of exceptional beauty, of Alaskan wilderness scenes.

For botanists and others interested in natural sciences, the names of most of the speakers participating in the Sixth Conference will have a familiar ring. All are thoroughly experienced ecologists, and their experience clearly points toward the need for preserving wilderness which can be used for observing natural biological phenomena. The kind of ecological knowledge derived through such observation is essential to caring for wilderness tracts now and in the years to come.

The opening address by Daniel B. Beard, "Plants and Animals in Natural Communities," emphasizes that the preservation of natural communities depends on man's ability to understand their complex ecological relationships. Fire, for example, can have disastrous effects but can also be beneficial in some communities, once this quality is perceived, by causing seed germination and favoring the development of tree seedlings. A distinctive wild land or wilderness literature is considered a requisite in order to convey the importance of preservation to modern society. Stanley A. Cain, in his "Ecological Islands as Natural Communities," believes that greater understanding on the part of the public of plants and animals as they are interrelated at the community level will enhance a fuller sense of appreciation in the minds of the individuals who come to enjoy the wilderness. Such understanding is fundamental to deepening man's conservation sense. Luna B. Leopold's "Ecological Systems and the Water Resource" brings out the oftentimes overlooked fact that environments are not static, and that reference points are needed in order to distinguish and evaluate natural changes. He cites the United States Geological Survey's interest in establishing so-called "bench mark" gauging stations which would measure streamflow from areas protected, as much as possible, from man's interference.

In "The Outlook for Conservation in Alaska," Robert Rausch is deeply concerned with the urgency for legislative measures to protect biota in our new State. He gives five points in need of attention by conservationists: (1) additional support for wildlife ranges and refuges, (2) protection of National Monuments, such as Katmai and Glacier Bay, against special interest groups by changing them to National Parks, (3) establishment of State parks, (4) protection of certain animals, such as polar bear and walrus, against more and more hunting, and (5) development of a program of conservation education in schools. Ian McTaggart Cowan's "Science and the Wilderness" was illustrated with slides, and it is unfortunate that these could not have been made part of the book. Cowan indicates that there is relatively little first-rate research on wilderness animal life, particularly large mammals, and believes that too many wildlife studies are superficial and overly concerned with managerial problems. The address by Raymond B. Cowles, "Population Pressure and Natural Resources," foresees the effect of growing numbers of people upon the National Parks whereby increasing impingement will eventually eliminate these natural areas entirely. He believes that in long-range planning, the need to take cognizance of this matter cannot be over-emphasized. Finally, Frank Fraser Darling's "Wilderness, Science, and Human Ecology" reiterates the need for a natural unaltered datum from which comparisons of "changed conditions caused by human interference" can be made. Darling shares the viewpoint stressed by other conference participants.

This book carries a grave message for everyone, not scientists alone, who is deeply concerned with the preservation of the wilderness. It should be heeded by every American but is of the utmost importance to administrators, planners, and public officials who are now doing the spadework for our civilization for the years to come. The service the Sierra Club is rendering through its leadership in conservation can never be fully appreciated in our time. But future generations will someday reflect with deep appreciation upon the work that the Club and conservationists are doing. C. J. HEUSSER, American Geographical Society, New York 32, N. Y.

Late-Pleistocene Environments of North Pacific North America. An Elaboration of Late-Glacial and Postglacial Climatic, Physiographic and Biotic Changes. Special Publication No. 35. By Calvin J. Heusser. 308 pp. Illus. Paper, American Geographical Society, New York, 1960, $4; cloth, $6.

This is an extremely well documented and comprehensive description of events that shaped the environments and influenced the distribution of plants along the North Pacific Coast during the past 14,000 years. The reconstruction of environments from Late Glacial time to present is based on 78 pollen chronologies correlated over 21 degrees of latitude from Fort Bragg, California to Kodiak Island, Alaska. The vegetation changes reflected in the pollen sequences are thoroughly integrated with evidence from regional geological, climatological, and biological studies. Much of the relevant information from these fields is so recent that it is cited as personal communications. According to the author the study is best categorized as an investigation in historical plant geography; however, his discussions and conclusions merit the detailed attention of ecologists, meteorologists, geologists, anthropologists, and botanists in general as well as phytogeographers and palynologists.

The text is divided into ten chapters. The first six chapters are concerned with a detailed discussion of the regional physiographical, climatological, and vegetational background, a review of the field of palynology, and a description of the author's methodology. Chapter 5, in which palynology is described and reviewed, will be of particular interest to botany and geology professors who have included or wish to include palynology in the content of their courses. Chapter 7 contains the author's analytical data: the description and illustration of the peat and pollen stratigraphy of 78 bog bores. Sixty-seven of the sections are new to the literature. The 11 sections previously reported on by Dr. Heusser are re-interpreted and integrated with his more recent data. Chapters 8 through 10 sum up the author's interpretation of his pollen profiles in terms of the environments deduced from the vegetation changes and the relationship of the vegetation changes to climatic and physiographic events. The ecological amplitude and survival relationship of various elements in the late-Pleistocene vegetation are discussed in terms of their probable migration from glacial refugia. Finally, the bearing of the interpretations on questions concerning man's migrations during the late-Pleistocene is examined. Two appendices are included after the text. Appendix A notes the location and briefly describes the physical and floristic features of the bogs sampled. Appendix B describes the physiography and geography of the North Pacific Coast province by province. It is recommended that Appendix B be read after the Foreword, written by Dr. H. P. Hansen, and before the author's abstract. Abbreviations of measurement and identification terms used are listed facing the author's abstract and a glossary of the technical terms follows the appendices.

Among the numerous conclusions arrived at by Dr. Heusser, two are of fundamental importance to an understanding of late-Pleistocene pollen stratigraphy and phytogeography:

(1) The apparently world-wide contemporaneity, though not necessarily synchroneity, of Late Glacial and Postglacial floristic changes. Particularly well documented in Dr. Heusser's data is a definitive floristic expression of a period (the Hypsithermal) beginning about 8000 years ago and ending about 3000 years ago during which the mean average annual temperature in temperate zones was higher than it is at present. With increase in latitude the Hypsithermal begins later and ends sooner but it can be seen in vegetation chronologies from areas as widely distant as Japan, New Zealand, Fuego Patagonia, The British Isles, Nova Scotia, Scandinavia, Northern Europe, etc.

(2) Accumulated evidence from glacial geology and physiography together with the new palynological data lend increasing support to the Hulténian postulate of ice-free refugia in south-central Alaska and the archipelagoes of southeastern Alaska. With waning of the cordilleran glaciers, migrants from these refugia quickly established themselves on the deglaciated terrain.

As in any work of this magnitude there are points with which one can take issue and picayunish defects like the oft' repeated broken "W" type. Some of the author's definitions are too concise as, for example, that for "Eustasy". However, the reader is forewarned by the author that the definitions given are not inclusive. The index is not as comprehensive as one might wish for in a reference book. For example, there appears to be no way of finding the text references to Naptowne and Skilak glaciation,

Middleton Island, etc. Also, no mention is made that pollen chronologies from the Quebec-Labrador region, a study cited in the text, failed to show evidence of the Hypsithermal interval or a final revertance period. In light of the substantial evidence presented by Dr. Heusser's chronologies and confirmatory chronologies from widely separated areas, some explanation for the apparent lack of evidence of the Hypsithermal and climatic deterioration in the Quebec-Labrador region seems to be required.

Dr. Heusser's authoritative and comprehensive study will undoubtedly become a standard reference work for the late-Pleistocene of the Pacific Northwest. It should become a model of excellence for similar studies in other parts of the world. For biogeographers, palynologists, physiographers and glaciologists it is an essential addition to their personal library.—R. L. PIERCE, Socony Mobil Field Research Laboratory, Dallas Texas.

Oligocene Plants from the Upper Ruby River Basin, Southwestern Montana. By Herman F. Becker. Geological Society of America, Memoir 82, 127 pages. 1961.

A late Oligocene flora of 85 identified species from the Ruby River Basin in southwestern Montana is prosaically described in this memoir. The method of study follows the pattern of recent reports by various students of western North American Tertiary paleobotany. Accordingly, fossil entities are equated with their closest living counterpart. On this basis the Ruby River Basin flora is said to incorporate a wide array of communities ranging from lake and marsh species to typical desert scrub plants. Grouping of the fossil species reveals an almost equal representation in the flora of Arcto-Tertiary elements and Madro-Tertiary components. Climatic conditions suggested by the fossil flora favor a warmer environment with less of the extremes of today. Comparison with the Colorado Florissant flora shows a 40% specific identity whereas the similarity is only 13% with the Oregon Bridge Creek flora. Becker concludes that these data indicate an intermediate position in time and ecology for the Ruby River Basin flora.

The faults of this memoir are the same but of lesser magnitude than are those found in the author's Mormon Creek flora published in volume 107 of Palaeontographica. Vagueness in expression is disconcerting at times. Perhaps in an attempt to achieve brevity the author sacrificed continuity of thought and clarity. Identification mistakes constitute the other area for comment. Becker's new species reflect a preoccupation with his own material and a lack of attention to pertinent published data. One extremely irksome and meaningless practice is his use of the designation holotype for unknown plants and animals (*e.g.*, Pl. 7, figs. 12, 13; Pl. 12, fig. 9; Pls. 31, 32). It is unacceptable procedure to use the term holotype if the organisms are neither described nor named. Any eventual valid holotype will not be based on these specimens unless at that time these are found to be the best representative of the new form. However, even then the credit will be given to the new author.

The assignment of names to fossil leaves is sometimes difficult and often precarious. However, in certain cases I cannot refrain from indicating disagreement. The following are examples: *Smilax* (Pl. 11, figs. 9–11) should be re-examined to determine if it might not be *Clematis*. Leaves assigned to *Myrica* (Pl. 12, fig. 1) show a marked difference from those illustrated for comparison in that figure 5 has lateral secondaries terminating in a tooth. In figure 1 these lateral veins end in a sinus. This difference and the asymmetrical base suggest Sapindaceae. The venation of the leaves (Pl. 16, figs. 13–15) certainly does not suggest assignment to live oaks. It is difficult to consider the extremely fine marginal serration of the specimens illustrated in Plate 17 as being a typical feature of these oaks. Figure 5 of Plate 17, for example, suggests *Alnus*. I cannot accept the designation of *Cercidiphylum* as *C. elongatum* when all the illustrated material indicates *C. arcticum*. Care has not been exercised naming a new species of *Cercocarpus* (Pl. 22, figs. 2–6) and of *Populus* (Pl. 13). These forms will be found to belong to species established by Axelrod. Finally, it will be necessary for Becker

to explain Plate 13, fig. 1 in this memoir and Plate 26, fig. 9 in Palaeontographica, volume 107, where he described the Mormon Creek flora. There is no doubt that these photos are of the SAME specimen yet they carry different widely separated numbers in the University of Michigan collections and are indicated to have come from different localities.

The landscape photos are excellent. It seems excessive, however, to devote Plates 4–6 to irrelevant scenery and two plates to insects and fish.

The memoir is a contribution to our knowledge of the expanding framework of Tertiary floristics in western North America. The interior location of the flora aids in establishing it as a necessary addition to the literature whereby comparative data are made available. However, the usefulness of this study would be enhanced if it afforded greater confidence in its contribution on floral composition.—WAYNE L. FRY, Department of Paleontology, University of California, Berkeley 4, California.

INDEX TO AMERICAN BOTANICAL LITERATURE

COMPILED BY

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WITH THE COLLABORATION OF

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TAXONOMY, PHYLOGENY, AND FLORISTICS

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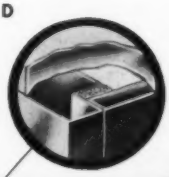

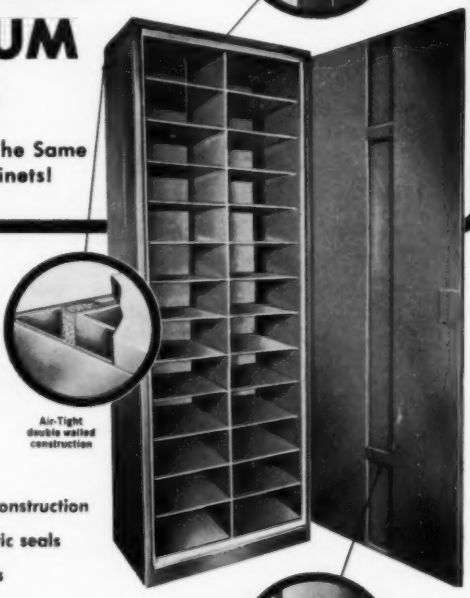

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